

REMARKS

This paper is filed in response to the official action dated May 19, 2005. This paper is timely-filed, as it is accompanied by a petition for an extension of time to file in the third month and a check covering the requisite fee of \$1020.00.

Claims 1-49 are pending, but claims 6, 8, 9, 18, 19, 22-25, 30-32, 36-47, and 49 have been withdrawn from further consideration. Claims 1-5, 7, 10-17, 20, 21, 26-29, 33-35, and 48 are presently at issue.

Claims 1-5, 7, 10-17, 20, 21, 26-29, 33-35, and 48 have been rejected under 35 U.S.C. §112, first paragraph, as assertedly failing to comply with the written description requirement. Additionally, claims 1, 4, 10, 13-15, and 28 have been rejected under 35 U.S.C. §102(b) as assertedly being anticipated by Coller, "Platelet GPIIb/IIIa Antagonists: The First Anti-Integrin Receptor Therapeutics," *J. Clin. Invest.*, 99(7):1467-1471 (1997).

By the foregoing, claim 1 has been amended without prejudice or disclaimer. Support for the amendment may generally be found throughout the application as filed. More specifically, support may be found at page 5, lines 24-27 of the present application. No new matter has been added.

In response to the examiner's comments regarding claims 35 and 48 at page 4 of the official action, Applicant submits that the claims were amended (in the previous response) such that they did not recite "wherein the first molecule is selected from the group consisting of the proteins set forth in Table 1."

The claim rejections are addressed below in the order presented in the official action. Reconsideration of the application is solicited in view of the following remarks.

CLAIM REJECTIONS – 35 U.S.C. §112, FIRST PARAGRAPH

Claims 1-5, 7, 10-17, 20, 21, 26-29, 33-35, and 48 have been rejected under 35 U.S.C. §112, first paragraph, as assertedly failing to comply with the written description requirement. Applicant traverses the rejections and respectfully submits that claims 1-5, 7, 10-17, 20, 21, 26-29, 33-35, and 48 are supported by an adequate written description.

The examiner recognized that the application "describes working examples of [17] first molecules as claimed," but asserted that the "17 species of first molecules described do not adequately describe the generic claims." *See* official action at pages 3 and 4. The examiner further asserted that "[d]escription of methods using 17 proteins in the

specification does not comprise a representative number of the undefined large genus of claimed methods.” *Id.*

Moreover, with regards to the elected species of ftsZ, the examiner alleged that:

the specification does not describe an allosteric site in FtsZ, or effectors of any type of FtsZ. The specification does not describe the Rossman fold structure of FtsZ. ... The specification does not describe a method utilizing FtsZ that comprises effectors, or the claimed structural limitations of FtsZ such as Rossman folds, or the claimed structural and functional limitations of the effector such as increasing or decreasing binding or enzymatic activity....

See official action at page 4.

Statutory law requires that the specification shall contain a written description of the invention. See 35 U.S.C. § 112, first paragraph. The courts have interpreted that provision as requiring that the description of the invention be sufficient to allow one of skill in the art to recognize that Applicant was in possession of the subject matter claimed. Vas-Cath v. Mahurkar, 935 F.2d 1555 (Fed. Cir. 1991); accord, M.P.E.P. § 2163 (I).

Possession is shown by describing the claimed invention with all of its limitations using descriptive means such as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. Possession may be shown by describing an actual reduction to practice or by describing distinguishing identifying characteristics sufficient to show that Applicant was in possession of the claimed invention. See, e.g., M.P.E.P. § 2163 (I).

In the "Summary of the Invention," Applicant provides the following description of the claimed subject matter:

...the present invention provides methods of modulating binding interaction between a first molecule which is not LFA-1 or an I domain-containing fragment thereof, and a binding partner molecule, said first molecule comprising an α/β domain structure, said α/β structure comprising an allosteric regulatory site, said method comprising the step of contacting said first molecule with an allosteric effector molecule that interacts with said allosteric regulatory site and promotes a conformation in a ligand binding domain of said α/β structure that modulates binding between said first molecule and said binding partner molecule.

See application at page 3, line 28 – page 4, line 4.

The application discloses a large number of proteins that include an α/β domain structure (*see* Table I) and also teaches how to identify proteins including such a domain for use in the claimed methods, as demonstrated, for example in Table 3 (*see* Example 1). The scope of the claims is therefore not limited to those proteins disclosed in Table I; proteins therein are simply exemplified embodiments. Each α/β protein includes common structural features, *i.e.*, each includes an α/β domain structure comprising alpha helix and beta sheet structures. Additionally, most α/β domain structures include a Rossman or Rossmann-like fold. In this regard, the application specifically teaches that Rossman or Rossmann-like folds are found in many members of the alpha/beta domain superfamily:

[m]any members of the [alpha/beta domain] superfamily, including proteins comprising an integrin I domain, von Willebrand factor comprising A domain structures, and various enzymes, have an open twisted beta sheet which gives rise to a fold in the protein's three dimensional structure. This fold is commonly referred to as a Rossmann fold, a Rossmann-like fold, or a dinucleotide binding fold.

See application at page 1, lines 22–26.

The aforementioned common structural characteristics of α/β proteins corroborate Applicant's possession of the claimed genus of methods as of the application filing date. *See, e.g.*, M.P.E.P. § 2163 (I). In view of the aforementioned application teachings, which provide distinguishing identifying characteristics for proteins of use in the claimed genus of methods, it is respectfully submitted that one of ordinary skill in the art would recognize that Applicant was in possession of the necessary common attributes or features possessed by α/β proteins for use in the claimed methods.

Furthermore, the examiner recognized that Applicant described the actual reduction to practice of the claimed methods using at least 17 α/β proteins.¹ In addition to the proteins acknowledged by the examiner, Applicant respectfully submits that example 15 of the application describes the actual reduction to practice of von Willebrand factor, and that example 18 of the application describes the prophetic reduction to practice of DapB, ENR, ERA-GTPase, and yihA.² In view of the aforementioned exemplified proteins, which each possesses the distinguishing identifying characteristics discussed

¹ The examiner indicated that the specification describes a working example using HPPK in Example 19. *See* page 4 of the official action. However, the examiner also indicated that Example 19 does not provide results that support the claimed method. *See* page 5 of the official action. Clarification is requested.

² A clarifying explanation supporting the examiner's contention that "Examples 15, 18, 19, and 20 do not provide results that support the claimed method" is respectfully requested. *See* official action at page 5.

above, it is respectfully submitted that one of ordinary skill in the art would recognize that Applicant was in possession of the necessary common attributes or features possessed by α/β proteins for use in the claimed methods.

To the extent the examiner is relying on The Regents of the Univ. of Cal. v. Lilly, 119 F.3d 1559 (Fed. Cir. 1997), Applicant submits that Lilly is off point with respect to the facts relating to the instantly claimed subject matter. The subject matter of the presently rejected claims relates to methods of modulating binding of α/β proteins and such proteins were known in the art as of the application filing date.³ In contrast, the rejected subject matter at issue in Lilly was a genus of polynucleotides encoding mammalian insulin, and the specification purportedly supported the claimed genus with the disclosure of a single polynucleotide encoding rodent insulin. Prior to disclosure of the rodent polynucleotide, no mammalian polynucleotide encoding insulin was known in the art. Therein lies the distinction between the facts in Lilly and the present application; in Lilly a genus of previously unknown compounds was being claimed, while here, use of known compounds is being claimed in a genus of methods.

Moreover, even if the decision in Lilly were on point, the Applicant has provided the requisite number of species to describe the genus of claimed methods. To the extent the examiner disagrees, Applicant respectfully requests that the examiner indicate how many species are representative and the basis for this determination.

With respect to the elected species of ftsZ, Applicant (prophetically) reduced to practice modulation of ftsZ binding to GTP in Example 20. Additionally, the application discloses that ftsZ is an α/β protein, and therefore the application teaches that ftsZ likely includes a Rossmann or Rossmann-like fold, as explained previously. Moreover, original claim 48, which is indirectly dependent from claim 5, recites that ftsZ comprises an allosteric regulatory site and a Rossmann fold structure. Further, original claim 48, which is indirectly dependent from claim 16, recites structural details regarding the Rossmann fold structure of ftsZ (i.e., the Rossmann fold in FtsZ comprises a β sheet having β sheet strands positioned in a 321456 orientation). Further, Table 3 in Example 1 includes data predicting that ftsZ includes an allosteric site based on the structural relatedness between ftsZ and molecules known to possess an allosteric site. The application further explicitly discloses that a Rossmann fold structure comprises an allosteric regulatory site (*see, e.g.,*

³ Applicant notes, however, that compounds identified or produced at some future date which possess the required structural limitations are also within the scope of the claims.

application at page 9, lines 15-16). Thus, the application teaches that ftsZ includes an allosteric site and a Rossman fold structure.

To the extent that the examiner continues to maintain that the present disclosure is lacking relative to ftsZ, Applicant directs his attention to Löwe *et al.*, *Nature*, 391:203-206 (1998) (copy attached hereto as Attachment A), which predates Applicant's application filing date. The Löwe reference discloses that ftsZ has a Rossman fold, and that the β sheet thereof has strands positioned in a 321456 orientation. Applicant maintains that it was within the purview of one of ordinary skill in the art to identify the attached reference as of the application filing date.

Applicant also disclosed that the claimed methods use effector molecules such as diaryl compounds, more preferably diaryl sulfide compounds and diarylamide compounds, and most preferably diaryl sulfide compounds. *See* specification at page 15, lines 2-9. The application discloses numerous specific diaryl compound structures. *See, e.g.*, specification at Table 2. Thus, the application discloses effector molecules of ftsZ.

For all of the foregoing reasons, Applicant submits that the claimed subject matter is supported by an express description of the full scope of the invention and one of skill in the art would recognize that Applicant possessed that subject matter at the time of filing. Accordingly, the rejection of claims 1-5, 7, 10-17, 20, 21, 26-29, 33-35, and 48 as lacking written description has been overcome and should be withdrawn.

CLAIM REJECTIONS – 35 U.S.C. §102

Claims 1, 4, 10, 13-15, and 28 have been rejected under 35 U.S.C. §102(b) as assertedly being anticipated by Collier, "Platelet GPIIb/IIIa Antagonists: The First Anti-Integrin Receptor Therapeutics," *J. Clin. Invest.*, 99(7):1467-1471 (1997). Applicant respectfully traverses the rejections.

It is well-established that each and every limitation of a claimed invention must be present in a single prior art reference in order for anticipation to occur. *See, for example, C.R. Bard, Inc. v. M3 Systems, Inc.*, 157 F.3d 1340, 1349 (Fed. Cir. 1998). The standard for anticipation is one of strict identity. This standard has not been satisfied with respect to claims 1, 4, 10, 13-15, and 28.

Collier describes a monoclonal antibody fragment antagonist of the platelet glycoprotein GPIIb/IIIa. The Fab fragment of the mouse/human chimeric monoclonal antibody 7E3 has also been found to inhibit $\alpha_v\beta_3$. The monoclonal antibody fragment is often referred to as "abciximab."

Under the appropriate stimuli, the platelet glycoprotein IIb/IIIa receptor binds fibrinogen to cross-link platelets (and thereby facilitates platelet aggregation) in the normal course. The monoclonal antibody fragment, abciximab, has been shown to inhibit platelet aggregation and thus platelet thrombus formation. Despite this disclosure, nothing in the reference unequivocally demonstrates that there is an allosteric interaction between glycoprotein IIb/IIIa receptor and abciximab, and various documentary evidence suggests that abciximab binds to the active ligand binding site. See, e.g., Bittl, *New England J. Med.*, 17:1290-1302 (1996), and Lefkovits *et al.*, *New England J. Med.*, 332:1553-1559 (1995) (copies attached hereto as Attachments B and C, respectively).

In this regard, each of the claims recites an *allosteric regulatory site*, which is different than an active ligand binding site. For example, the present application discloses that allosteric sites are distinguishable from ligand binding sites at page 5, lines 19-27.

Furthermore, claims 1, 4, 10, 13-15, and 28 recite an allosteric effector small molecule. Monoclonal antibody fragments such as abciximab are not small molecules.

For the reasons set forth above, it is respectfully submitted that the outstanding anticipation rejections of claims 1, 4, 10, 13-15, and 28 have been overcome and therefore should be withdrawn.

CONCLUSION

It is submitted that the application is in condition for allowance. Should the examiner wish to discuss any matter of form or procedure in an effort to advance this application to allowance, he is respectfully invited to telephone the undersigned attorney at the indicated telephone number.

Respectfully submitted,

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Correspondence and requests for materials should be addressed to E.N. Coordinates referred to in this Letter have been deposited in the Brookhaven Protein Data Bank with ID 1tub and will be accessible within one year.

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Two FtsZ genes (named after filamenting temperature-sensitive mutant Z) from the archaeon *M. jannaschii* have been characterized by the genome project¹¹. One gene, MJ0370, was amplified by genomic polymerase chain reaction (PCR) and expressed in *E. coli*/C41, a mutant of BL21 capable of expressing toxic genes¹². Proteolysis during cell disruption was minimized by using heat-shock treatment. Cubic crystals were obtained and the structure was solved by multiple isomorphous replacement and density modification (see Methods and Table 1). The model (Fig. 1) contains residues 23–356, 116 water molecules, and one molecule of GDP; weak density for residues 1–22 was visible as an extension from helix H0.

together with the arrangement of five helices (H1, HL1, H2, H3 and H4), is consistent with typical Rossmann-fold topology¹⁴. Helix H2A is unique to FtsZ. Numbering of secondary structure elements (Fig. 2) follows the corresponding elements of p21^{ras} proteins.

The C-terminal domain, spanning residues 228–356, consists of a mainly parallel four-stranded central β -sheet supported by two helices on one side. The topology of the sheet is 1423, with strand 4 antiparallel to the others. The uncovered side of the sheet makes contacts with helix H5 and is otherwise open to the solvent. The fold of the C-terminal domain is related to chorismate mutase of *Bacillus subtilis* and can be superimposed on PDB entry 1COM¹⁵ with an r.m.s. deviation of 1.83 Å over 52 C α atoms (SC1, HC2, SC2, HC3, SC3 and SC4). Additionally, sequence comparisons give similarities to calmodulins in three loop regions (Swissprot CALM-TRYCR; loops between H5/HC1, SC1/HC2, and SC2/HC3) and to adenylyl cyclase (CYA1_HUMAN; residues 620–740), making a role in calcium-binding feasible. The electrostatic potential on the

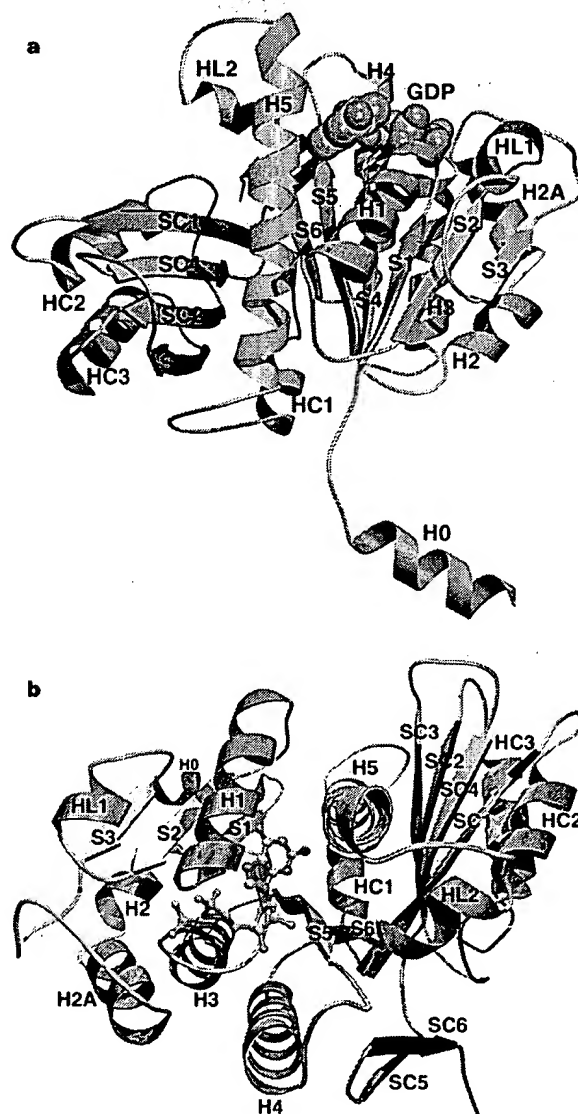


Figure 1 Ribbon drawings of FtsZ (residues 23–356) from *M. jannaschii*. **a**, View showing the GTPase domain in blue/green, the C-terminal domain in red/orange, and the connecting helix H5 in yellow. GDP is represented by a space-filling model. **b**, View of FtsZ rotated by $\sim 90^\circ$ from that in **a**. GDP is represented by a ball-and-stick model. Figures were prepared with POVSCRIPT (D. Peisach, personal communication)²⁸

surface of the C-terminal domain is dominated by a large patch of acidic residues on the open side of the β -sheet. This region forms crystal contacts to partly disordered residues 1–10 of a symmetry-related molecule. Residues 343–352 at the C terminus form a small β -hairpin which contacts S5 and H4 of the GTPase domain. Residues 357–372 are disordered in the crystal. The electron density for the C-terminal domain is slightly weaker than for the GTPase domain, probably because the C-terminal domain has only a few crystal contacts.

As predicted^{16,17}, GDP binding to FtsZ is different from typical GTPases¹⁸, although the GTPase domain of FtsZ is related to the fold of typical GTPases like p21^{ras}. Six distinct sequence regions in FtsZ make contacts to the nucleotide: loops T1–T4 (tubulin loops) and two regions for sugar binding and guanine binding, respectively (Fig. 2). The GTPase domain starts with the typical strand–loop–helix motif between S1 and H1, but the conformation of this loop is different from that in classical P-loop proteins. FtsZ makes three additional contacts to the nucleotide's phosphates: loop T2 between

S2 and HL1, loop T3 between S3 and H2A, and loop T4 between S4 and H3 carrying the tubulin signature motif. All four loops make mainly backbone contacts to the phosphates. As FtsZ was crystallized in the presence of EDTA, we did not expect to find a magnesium ion complexed to GDP. A poorly ordered water molecule (WAT472) occupies a similar position to the magnesium ion in p21^{ras}–GDP¹³; however, WAT482 is complexed between the β -phosphate and Asn 70, which makes it unlikely that this position in FtsZ would be occupied by a positively charged magnesium ion. Furthermore, it has been reported that nucleotide binding to FtsZ, but not nucleotide hydrolysis, is independent of magnesium¹⁹. The structure displays a pocket for the γ -phosphate and we found that fresh crystals showed weak electron density for a γ -phosphate. We think it is possible that GTP, originally bound to the protein, is slowly hydrolysed in the crystals. We were not able to detect large conformational changes between new and old crystals but the molecule may be fixed in one state by crystal restraints. The loop between S5 and H4 contains two residues that specifically bind the sugar moiety of the nucleotide (Fig. 3): Glu 165 hydrogen-bonds with the two hydroxyl groups O2' and O3' and Arg 169 makes contacts to the O5' hydroxyl and the α -phosphate. Guanine recognition is mainly accomplished by residues within H5: Asp 212 points to N1 and Phe 208 stacks on the aromatic ring. This aromatic stack is extended by Phe 162 and Phe 196. Leu 215 makes a hydrophobic contact on the other side of the guanine.

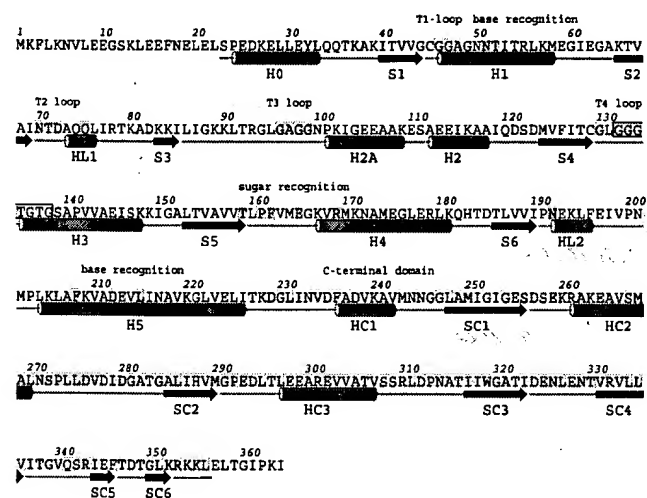


Figure 2 Secondary structure assignment of FtsZ1 from *M. jannaschii*. The secondary structure of FtsZ determined from the crystal structure was calculated with DSSP²⁹. The numbering of secondary structure elements follows the p21^{ras} nomenclature¹³. Elements in the C-terminal domain are numbered SC and HC for strands and helices, respectively. Residues highlighted in yellow make contact with GDP. Residues of the C-terminal domain are shaded. Prepared using ALSRIPT³⁰.

Table 1 Crystallographic data

Space group / 2,3 (199)	NAT12	NAT11	Thiomersal (3 mM)	
			EMTS1 (48 h)	EMTS3 (18 h)
Temperature	100 K	RT	RT	RT
Resolution (Å)	2.8	3.7	4.0	4.2
Completeness (%)	99.0 (99.7)	97.3	90.2	97.1
R_{merge}	0.065 (0.31)	0.12	0.14	0.15
$I/\sigma(I)$ last shell	2.3	1.7	1.6	2.1
R_{iso}			0.19	0.16
Phasing power†			1.88	1.83
Refinement	Residues 23–356, 1 GDP and 116 water molecules			
Model	NAT12, 8–2.8 Å, all reflections			
Data	0.199 (R_{free} 0.282)			
R factor	Bonds: 0.012 Å, angles: 1.77°; temperature factors: 3.4 Å ²			
R.m.s. deviations				

Completeness and R_{merge} for the outermost shell (2.80–2.95 Å) are in parentheses for NAT12. $R_{\text{merge}} = \sum_i \sum_j |I(h,i) - \langle I(h,i) \rangle| / \sum_i \sum_j I(h,i)$ where $I(h,i)$ are symmetry-related intensities and $\langle I(h,i) \rangle$ is the mean intensity of the reflection with unique index h .

† $R_{\text{iso}} = 2 \sum_i |F_{\text{PH}} - F_{\text{P}}| / \sum_i (F_{\text{PH}} + F_{\text{P}})$ where F_{PH} and F_{P} are the derivative and native structure factor amplitudes, respectively.

‡ Phasing power: mean value of the heavy-atom structure amplitudes divided by the residual lack-of-closure. Figure of merit was 0.59 and 0.42 for centric and acentric reflections, respectively.

§ Five per cent of reflections were selected for determination of free R factor.

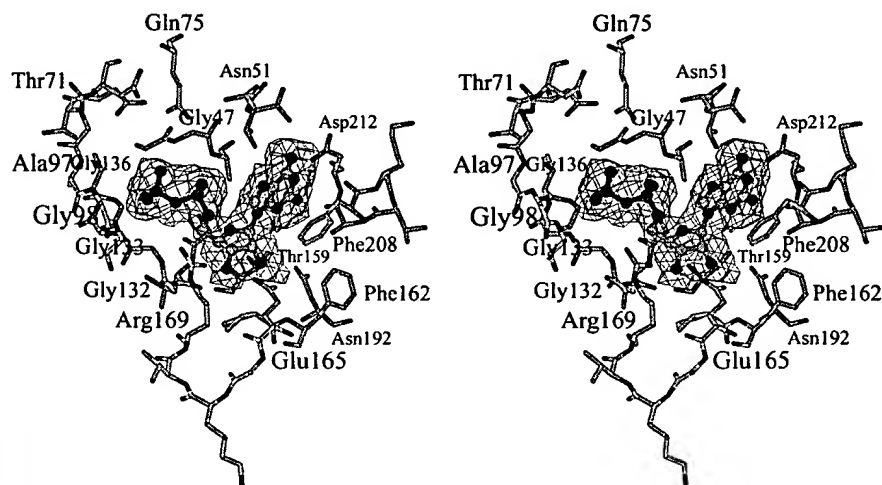


Figure 3 Stereo plot of the active site of FtsZ. Superimposed is the final $2F_o - F_c$ electron density map for the nucleotide contoured at 1σ . Prepared with MOLSCRIPT²⁸.

Asn 51 forms a hydrogen bond with O6 of the base. By contacting H5, the nucleotide may induce a conformational change during hydrolysis, moving the relative orientation of the catalytic domain and the C-terminal domain. A region around Gly 221 may deliver the flexibility between the two β -sheets for this type of movement. As in other GTPases, no residue could be identified that provides an activated nucleophile for hydrolysis. A comparison between the active sites of FtsZ and p21^{ras} in the GDP-bound state¹³ reveals important differences: the P-loop in p21^{ras} (G1 box, residues 10–17) has no counterpart in FtsZ but is probably functionally related to loops T1 and T4 in FtsZ. Residues in T1 and T4 make backbone contacts to the phosphates (residues 44–48 and 130–136, containing the tubulin signature motif). Thr 135 of FtsZ is approximately in the same position as Ser 17 of p21^{ras}. The T2 loop in FtsZ (residues 70–75) is approximately in the same place as the residues of the G2 box of p21^{ras} (residues 32–37). Asp 72 of FtsZ is the only acidic side chain in the active site of FtsZ and may be the equivalent of Asp 57 of p21^{ras}. Loop T3 in FtsZ (residues 94–99) is nearly in the same place as G box G3 of p21^{ras} (residues 57–60) with respect to the phosphates. Residues Lys 117 and Asp 119, located in a loop between S5 and S6, make the most important contacts to the guanine ring in p21^{ras}. The equivalent residues in FtsZ are Phe 208 and Asp 212, respectively. These residues are located in helix H5 in FtsZ. The sugar moiety in FtsZ is rotated by $\sim 180^\circ$ with respect to p21^{ras} if the central β -sheet of the two proteins is aligned. The residue making the hydrogen bonds to O2' and O3' in p21^{ras} is located in the first half of the sequence (p21^{ras} Asp 30), whereas the corresponding residue in FtsZ (Glu 165) is located in the second half of the GTPase domain in the sugar-binding loop (residues 159–169). Sequence comparisons between tubulins and FtsZ had already revealed limited homology of ~ 10 – 18% identity^{7,19,20}. Sequence alignment based on the structure of FtsZ alone (data not shown) revealed that most residues involved in GDP binding and several structural glycines and prolines are conserved and made it likely that the GTPase domain of tubulin is a Rossmann fold as well. The C-terminal domain of tubulins is larger and has very limited sequence homology to FtsZ, possibly reflecting diverse differences between the variety of interacting molecules. Strong structural similarity between FtsZ and tubulin both in the GTPase and the C-terminal domain is now evident from comparing the structure of FtsZ with the two-dimensional crystal structure of α - and β -tubulin¹⁰. Tubulin lacks the N-terminal extension of FtsZ but contains a large insertion between H1 and S2. Two large helices in tubulin extend the C-terminal domain. Nucleotide binding is very similar between FtsZ and β -tubulin, with many residues involved in GDP binding being conserved.

The 22 partly disordered N-terminal residues stick out of the molecule and make important crystal contacts, as do the last visible residues at the C terminus, which form a twofold contact with symmetry-related residues of another molecule. Inspection of the crystal packing reveals no obvious protofilament structures, as expected for a crystal packing with one molecule per asymmetric unit and a high-symmetry space group. The exposed N terminus with ~ 33 residues sticking out of the compact GTPase domain is rapidly degraded by proteases in the C41 expression host. Comparison between different FtsZ sequences reveals that this N-terminal extension is highly variable and is very short in *E. coli*, where it starts at residue 28, so it is unlikely to be important in filament formation. The C terminus is visible up to residue 356, with eight residues of the wild-type protein and the additional eight residues of the His₆-tagged protein being disordered. The C termini of FtsZ sequences are divergent from residue 342 onwards, after the last sheet of the C-terminal domain. Both the C and N termini contain many acidic residues and acidic/hydrophobic repeats. These sequences could simply enhance the solubility of monomers or filaments, although other functions have been proposed for the C termini of tubulins²¹. FtsZ(MJ0370) from *M. jannaschii* contains two cysteines, Cys 129

and Cys 45, which lie near the active-site strands S1 and S4 in the GTPase domain: this geometry would allow formation of a disulphide bridge and one mercury atom can bind to both residues. Because of its high similarity to tubulin, FtsZ should prove to be a simple model system for microtubule dynamics and be amenable to protein engineering. □

Methods

Protein expression, purification and crystallization. Genomic DNA was extracted from a living culture of *M. jannaschii* (DSM 2661, ATCC 43067) with a commercial kit (Promega Wizard). Genomic PCR using two primers (5'-GAAGTCCCATATGAAATTTCTAAAAACGTTTTA-3', 5'-CGTATTAGGATCCAATTTTGGAAATTCCTGTGTGTTCTA-3'), replacing the GTG start codon with ATG, produced a 1,115-bp DNA carrying the complete 1,092 bp of MJ0370 and unique cleaving sites for *NdeI* and *BamHI*. This fragment was cloned into the pHis17 vector (B. Miroux, personal communication), putting it under control of a T7-promoter and adding eight residues at the C terminus (GSHHHHHH) to yield a protein of 372 residues. C41 cells¹² were transformed and expressed the His-tagged protein after addition of IPTG. After induction in log phase, cells from a 10-litre culture were collected and snap-frozen in liquid nitrogen. The frozen pellet was powdered under liquid nitrogen and poured directly into 200 ml of boiling buffer A (50 mM Tris-HCl, 300 mM NaCl, pH 8.0) and stirred for 90 s. After addition of ice to a final volume of 400 ml, the lysate was centrifuged and applied to a Ni-NTA column (Qiagen). The FtsZ protein eluted at ~ 400 – 450 mM imidazole in buffer A, pH 6.0. The protein was further purified on a Sepharose-6B column (Pharmacia) using 20 mM Tris-HCl, 1 mM EDTA, 1 mM Na₂S₂O₃. FtsZ eluted as an oligomer under low-salt conditions. The product was checked by electrospray mass spectrometry (observed: 39,889.12 g mol⁻¹; estimated: 39,891.33 g mol⁻¹). Crystals were grown by the hanging-drop vapour-diffusion technique using 0.1 M MES pH 6.62, 17% PEG400 and 6% ethanol as crystallization solution. Droplets composed of 3 parts of 10 mg ml⁻¹ protein solution and 1 part crystallization solution were equilibrated for a minimum of one week. Two different crystal forms grew under these conditions, distinguishable by the lack of birefringence for one of them. These latter crystals belong to cubic spacegroup *I*2₁3, with one molecule per asymmetric unit and 71% solvent content; cell dimensions are $a = b = c = 159.14$ Å, with all angles 90° . Crystals for multiple isomorphous replacement were collected in crystallization solution and mounted in sealed capillaries. A crystal for the frozen dataset NAT12 was collected in crystallization solution and soaked for 5 min in 0.1 M MES pH 6.62, 25% PEG200 and 6% ethanol and frozen in a stream of cold nitrogen at 100 K.

Structure determination and refinement. Each low-resolution dataset was collected from a single crystal, using a MAR imaging-plate detector mounted on an Elliot GX13 rotating anode X-ray generator. The final native dataset NAT12 was collected from a frozen crystal at beamline 9.6 at the Daresbury SRS. Images were indexed and integrated with the MOSFLM²² package and further processed using the CCP4 suite of programs²³. The structure was determined by the MIRAS method using only one derivative (ethylmercurithiosalicylic acid, thiomersal, 3 mM) with two different soak times of 18 and 48 h, respectively. Heavy-atom positions were determined using SHELXS²⁴ and initial phases were calculated with MLPHARE. Phasing statistics are given in Table 1. After 20 cycles of phase refinement using SOLOMON²⁵ and phase extension from 4.0 to 3.7 Å resolution, a superb electron density map was obtained. Residues 1–356 could be built into the first density using FRODO²⁶, although the density for residues 1–22 was weak and these residues were later omitted from the final model. Crystallographic refinement was performed using the programs REFMAC (room-temperature data sets; phase-restrained) and X-PLOR²⁷. A total of 26 cycles of refinement and manual rebuilding was necessary to switch from the unfrozen to the frozen native dataset. At 2.8 Å resolution, solvent mask correction was applied and a resolution range of 8.0–2.8 Å was used. No reflections were excluded from refinement by their signal-to-noise ratio. The current model contains residues 23–356, 116 water molecules and 1 GDP molecule. Residues 1–22 gave weak difference density but were not refineable. The model shows good geometry with an r.m.s. deviation over bond length of 0.012, bond angles of 1.77° and no Ramachandran outliers. Individual temperature factors have been refined using tight restraints. They are higher for the C-terminal domain (residues 227–356), with an overall temperature

factor over residues 23–356 of 53 Å² (65 Å² from the Wilson plot). Coordinates and structure factors have been deposited in the Brookhaven Protein Data Bank.

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Correspondence and requests for materials should be addressed to J.L. The identity codes for the FtsZ coordinates and structure factors in the Protein Data Bank are 1FSZ and 1RFSZF, respectively.

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Review Article

*Medical Progress*ADVANCES IN CORONARY
ANGIOPLASTY

JOHN A. BITTL, M.D.

THE goal of therapy in patients with coronary artery disease is to alleviate symptoms of angina and reduce the risk of death or nonfatal myocardial infarction. Although coronary angioplasty immediately reduces anginal symptoms in almost all patients who undergo it, its use is associated with death or nonfatal myocardial infarction in about 5 percent of patients¹⁻⁸ and with restenosis requiring repeated angioplasty or bypass surgery in about 30 percent.^{3-6,9} Recently, several clinical trials have shown that the implantation of coronary stents^{5,6} or treatment with blockers of platelet glycoprotein IIb/IIIa receptors¹⁰⁻¹² reduces the occurrence of acute complications and restenosis in patients undergoing coronary angioplasty. These new therapies have spread rapidly and have changed the practice of interventional cardiology remarkably since 1994, when the topic was last reviewed in the *Journal*.¹³

Advances in coronary angioplasty have not occurred in isolation. There have also been improvements in the medical and surgical treatment of coronary artery disease, along with new insights into the natural history of coronary atherosclerosis. An update on coronary angioplasty is thus incomplete without attention to the benefits of new medical and surgical therapies. The aim of this review is to identify which patients will derive the greatest benefit from the various cardiovascular therapies available.

DEVICES FOR CORONARY
REVASCULARIZATION

The growth of interventional cardiovascular procedures has been staggering (Fig. 1). Almost 900,000 coronary angioplasty procedures were performed

worldwide in 1995.¹⁴ Since 1994, the use of balloon angioplasty has leveled off, while the use of coronary stents has grown substantially. The number of stent-implantation procedures is expected to exceed that of conventional balloon angioplasty procedures by the year 2000.¹⁴

Balloon Angioplasty

Balloon catheters are used as the sole devices in percutaneous transluminal coronary angioplasty (PTCA), or "balloon angioplasty," and as adjunctive dilating devices in most other interventional procedures. Since 1994, balloon catheters have undergone technical refinements. The deflated profile of 3.0-mm balloon catheters has been reduced from 0.037 in. (0.94 mm) to about 0.030 in. (0.76 mm). Improvements in catheter design have been partially responsible for higher success rates in recent years despite the older age of the patients and the more frequent occurrence of unstable angina and multivessel coronary artery disease in patients undergoing PTCA than in the past (Table 1).

Although the efficacy of PTCA has improved, it does not consistently result in a large lumen in the dilated vessel. This is important, because arteries with larger lumen diameters have a lower risk of subsequent restenosis than incompletely dilated arteries (the observation being that "bigger is better").¹⁵ When oversized balloons are used to dilate coronary vessels, however, the risk of vessel dissection and ischemic complications increases.¹⁶ Thus, several approaches involving the removal of atheromatous tissue have been developed to overcome the limitations of PTCA.

Atherectomy and Laser Angioplasty

During directional coronary atherectomy, atherosclerotic tissue is extracted from the coronary artery with a cutting blade spinning at 5000 rpm in the tip of the atherectomy device. During excimer-laser angioplasty, light at a wavelength of 308 nm emitted from optical fibers at the catheter tip vaporizes atheromatous tissue. Although directional atherectomy and excimer-laser angioplasty usually result in larger lumen diameters than PTCA, these new treatments have not reduced the rates of acute complications or restenosis after coronary angioplasty.^{3,4,17-19} Some investigators have attributed the failure of directional atherectomy to achieve better clinical outcomes than PTCA to the fact that conservative cutting techniques have produced only a moderate increase in the final lumen diameter.²⁰

Concern about directional atherectomy was in-

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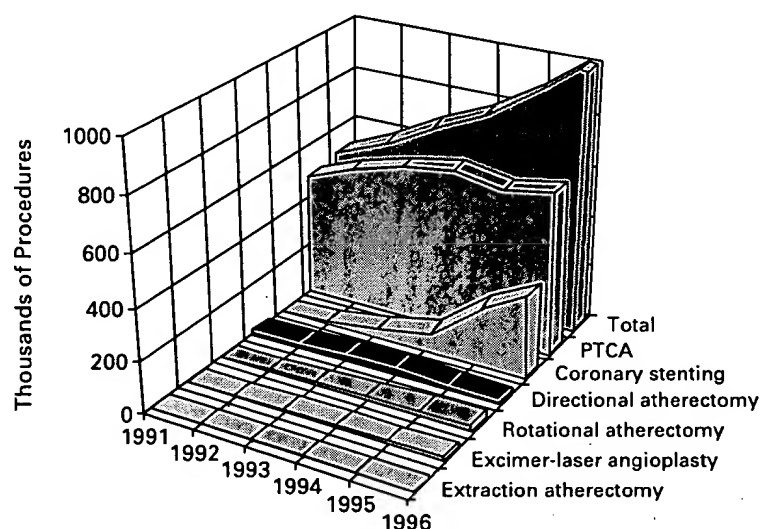


Figure 1. Rate of Growth in the Use of Interventional Cardiovascular Procedures from 1991 to 1996. The rates for 1996 are estimates. Data are from Lemaitre et al.¹⁴

creased by studies reporting that the rates of release of creatine kinase MB after the procedure (38.3 percent vs. 19.4 percent²¹) and one-year mortality (2.2 percent vs. 0.8 percent²²) were about twice as high as those observed after standard PTCA. These higher rates may be caused by embolization of atheromatous debris during an otherwise successful atherectomy procedure, leading to small myocardial infarctions.²¹ If modified techniques²³ involving more complete tissue removal and greater use of adjunctive PTCA result in clinical benefit, directional atherectomy may have a resurgence in popularity.

The failure of excimer-laser angioplasty to achieve better clinical outcomes than PTCA was also attributed to inadequate tissue removal,²⁴ along with an increased risk of vessel dissection²⁵ and perforation²⁶ from the formation of intraluminal vapor bubbles in blood.²⁷ The incidence of dissection may be reduced by infusing saline through the guide catheter during excimer-laser angioplasty.²⁸

Rotational atherectomy^{29,30} is another approach for removing atheromatous plaque from coronary arteries. This technique uses a diamond-studded burr spinning at about 180,000 rpm to excavate calcified or fibrotic plaque, allowing microscopic debris to embolize to the coronary capillary bed. No multicenter, randomized trials proving its superiority over PTCA have been reported. The Excimer Laser Rotational Atherectomy Balloon Angioplasty Comparison was a single-center study involving 615 patients who underwent rotational atherectomy, excimer-laser angioplasty, or PTCA.³¹ Although rotational atherectomy was associated with a higher short-term success rate than PTCA (90 percent vs. 80 percent), the rates

TABLE 1. INCREASING SUCCESS OF PTCA OVER TIME.*

VARIABLE	NHLBI ANGIOPLASTY STUDY I†	NHLBI ANGIOPLASTY STUDY II†	MAPS‡	MULTICENTER DATA BASE§
Years of study	1977–1981	1985–1986	1991	1990–1994
No. of patients	1155	1802	200	3787
Base-line characteristics				
Median age (yr)	54	58	62	61
Unstable angina (%)	37	49	52	63
Multivessel CAD (%)	25	53	100	51
Success (%)¶	61	78	90	87
Complications				
Death (%)	1.2	1.0	1.0	0.9
MI (%)	4.9	4.3	1.5	5.2
Emergency CABG (%)	5.8	3.4	1.0	2.7

*NHLBI denotes National Heart, Lung, and Blood Institute, MAPS Multivessel Angioplasty Prognosis Study Group, CAD coronary artery disease, CABG coronary-artery bypass grafting, and MI myocardial infarction.

†Data were obtained from Detre et al.⁸

‡Data were obtained from Ellis et al.⁷

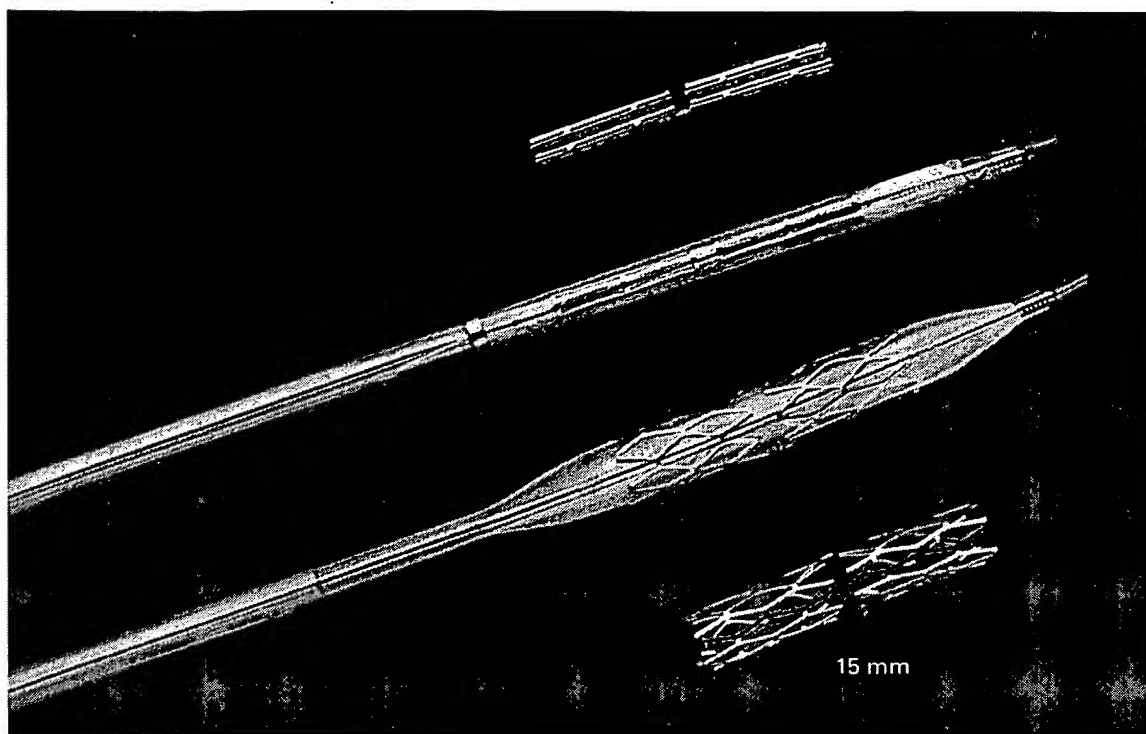
§Data were obtained from Ellis et al.¹

¶Success was defined as less than 50 percent residual stenosis or a 20 percentage point decrease in vessel narrowing at the target lesion without major complications (death, myocardial infarction, or emergency bypass surgery) during hospitalization.

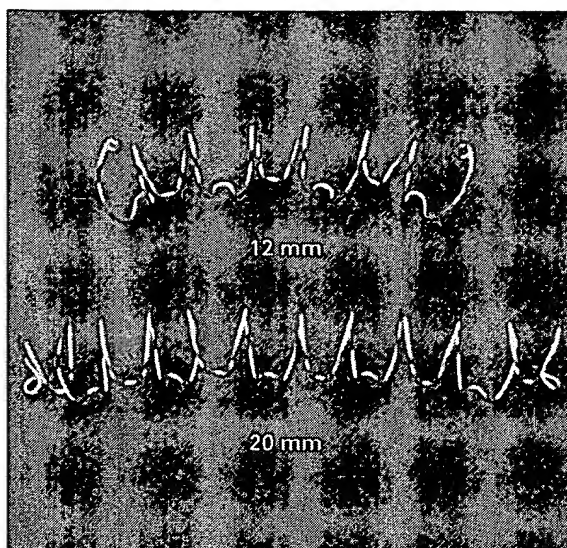
of major ischemic complications or repeated revascularization were higher six months after treatment (46 percent vs. 37 percent).³¹

Coronary Stenting

Coronary stents are fenestrated stainless-steel tubes that can be expanded by a balloon and provide a



A



B

Figure 2. Coronary Stents Approved for Use by the Food and Drug Administration.

Panel A shows a stainless-steel, slotted-tube, Palmaz-Schatz stent. An unexpanded stent is shown at the top of the panel, followed by an unexpanded stent mounted on a balloon catheter, an expanded stent on a fully dilated balloon, and an expanded stent with its slotted-tube configuration after deflation and removal of the balloon catheter. Although this stent is approved for use in reducing restenosis in native coronary arteries 3.0 mm or more in diameter, it is also widely used for other indications, such as abrupt vessel closure. Panel B shows balloon-expanded, stainless-steel, flexible-coil Gianturco-Roubin stents in 12-mm and 20-mm lengths. These stents are approved for abrupt vessel closure but have several other important uses as well.

scaffold within coronary arteries to treat acute vessel dissection and reduce the risk of restenosis. Two designs are currently approved by the Food and Drug Administration for general use (Fig. 2).

Current Applications

Coronary stenting reduces the immediate need for bypass surgery for abrupt vessel closure during PTCA.³²⁻³⁴ A mechanical treatment of abrupt closure seems appropriate, because this problem is predominantly caused by mechanical disruption, such as vessel dissection or plaque extrusion, in almost 80 percent of cases and by thrombus in about 20 percent of cases.^{2,35,36} When stenting is used to treat thrombus-containing lesions, however, the risk of ischemic complications increases.^{37,38}

Coronary stenting also reduces the likelihood of restenosis in particular groups of patients. Two multicenter, randomized trials showed that the incidence of restenosis was 25 to 30 percent lower after coronary stenting than after PTCA for new lesions in large native coronary arteries measuring 3.0 mm or more in diameter (Table 2).^{5,6,39} Late restenosis of coronary stents is rare. The lumen diameter of stented arteries did not decrease, according to serial angiographic observations made from six months to three years after the procedure.⁴⁰

The use of coronary stents has not been restricted to the prevention of restenosis or the treatment of abrupt vessel closure. This versatile therapy is commonly used on a provisional basis for residual narrowings or mild dissections after PTCA. Coronary stenting is used in high-risk situations unlikely to be successfully managed with conventional PTCA. For example, coronary stents are used to treat stenoses of the left main coronary artery in patients who cannot undergo bypass surgery (Fig. 3),⁴¹ stenoses in diseased saphenous-vein grafts,^{42,43} and total occlusions recanalized with PTCA or laser angioplasty.^{44,45}

Subacute Thrombotic Occlusion of Coronary Stents

The chief limitation of coronary stenting is subacute thrombotic occlusion, which occurs in about 4 percent of patients within 2 to 14 days after stent implantation and almost always results in a myocardial infarction or death.^{5,6,38,46-48} Subacute thrombotic occlusion after stent implantation is a more serious problem than complete vessel closure after PTCA. The latter problem also occurs in about 4 percent of patients but appears most commonly while the patient is still in the cardiac catheterization laboratory, where the problem can be treated. Moreover, only one third of patients with complete vessel closure after PTCA have major ischemic complications.²

Initial efforts to prevent stent-associated thrombosis involved an intensive anticoagulation regimen consisting of aspirin, dipyridamole, dextran, and

heparin during stent implantation and warfarin after the procedure. When bleeding and stent-associated thrombosis occurred simultaneously in some patients receiving anticoagulation therapy, it became clear that this approach did not prevent thrombosis. Using intravascular ultrasound imaging, Colombo and colleagues⁴⁹ showed that stents must be dilated after implantation with balloons at high pressures (up to 16 to 20 atmospheres). When stents are fully expanded, the risk of subacute thrombotic occlusion is low, even in patients who are not receiving anticoagulation therapy.⁴⁹ Because high-pressure balloon inflations are used in most cases to expand coronary stents, intravascular ultrasound imaging may not be needed routinely to document full stent expansion. In a pooled analysis, stent-associated thrombosis occurred in only 33 of 2630 patients (1.3 percent) who did not re-

TABLE 2. RESULTS OF MULTICENTER RANDOMIZED STUDIES COMPARING STENTS WITH CONVENTIONAL PTCA.*

END POINT	BENESTENT†		STRESS‡	
	PTCA (N = 257)	STENT (N = 259)	PTCA (N = 202)	STENT (N = 205)
	percentage of patients			
Early events (≤14 days)				
Success§	—	—	96.5	99.5¶
Stent thrombosis or vessel closure	1.5	3.4	2.7	3.5
Death	0.0	0.0	1.5	0.0
MI	3.1	3.5	5.0	5.4
CABG	1.5	3.1	4.0	2.4
Events up to 6-7 mo				
Death	0.8	0.4	1.5	1.5
MI	4.7	4.3	6.9	6.3
CABG	4.3	6.2	8.4	4.9
Repeated angioplasty	23.3	13.5¶	12.4	11.2
Angiographic restenosis	32.0	22.0¶	42.1	31.6¶
Events up to 1 yr				
Death	0.8	1.2	—	—
Stroke	0.8	0.4	—	—
MI	5.1	5.4	—	—
CABG	5.8	8.1	—	—
Repeated angioplasty	26.8	17.8¶	—	—
Any event	38.9	32.0	—	—

*All patients treated with stents were given warfarin anticoagulation therapy. Benestent denotes Belgium-Netherlands Stent Study, STRESS Stent Restenosis Study, MI myocardial infarction, and CABG coronary-artery bypass grafting.

†Data were obtained from Serruys et al.⁵ and Macaya et al.³⁹ The patients in the study had stable angina with stenoses in native coronary arteries ≥3.0 mm in diameter.

‡Data were obtained from Fischman et al.⁶ The patients had stable or unstable angina with stenoses in native coronary arteries ≥3.0 mm in diameter.

§Success was defined as less than 50 percent residual stenosis without major complications (death, myocardial infarction, or emergency bypass surgery) during hospitalization.

¶P<0.05 for the comparison with PTCA.

||This was defined as evidence of stenosis of more than 50 percent on coronary arteriography more than four months after treatment.

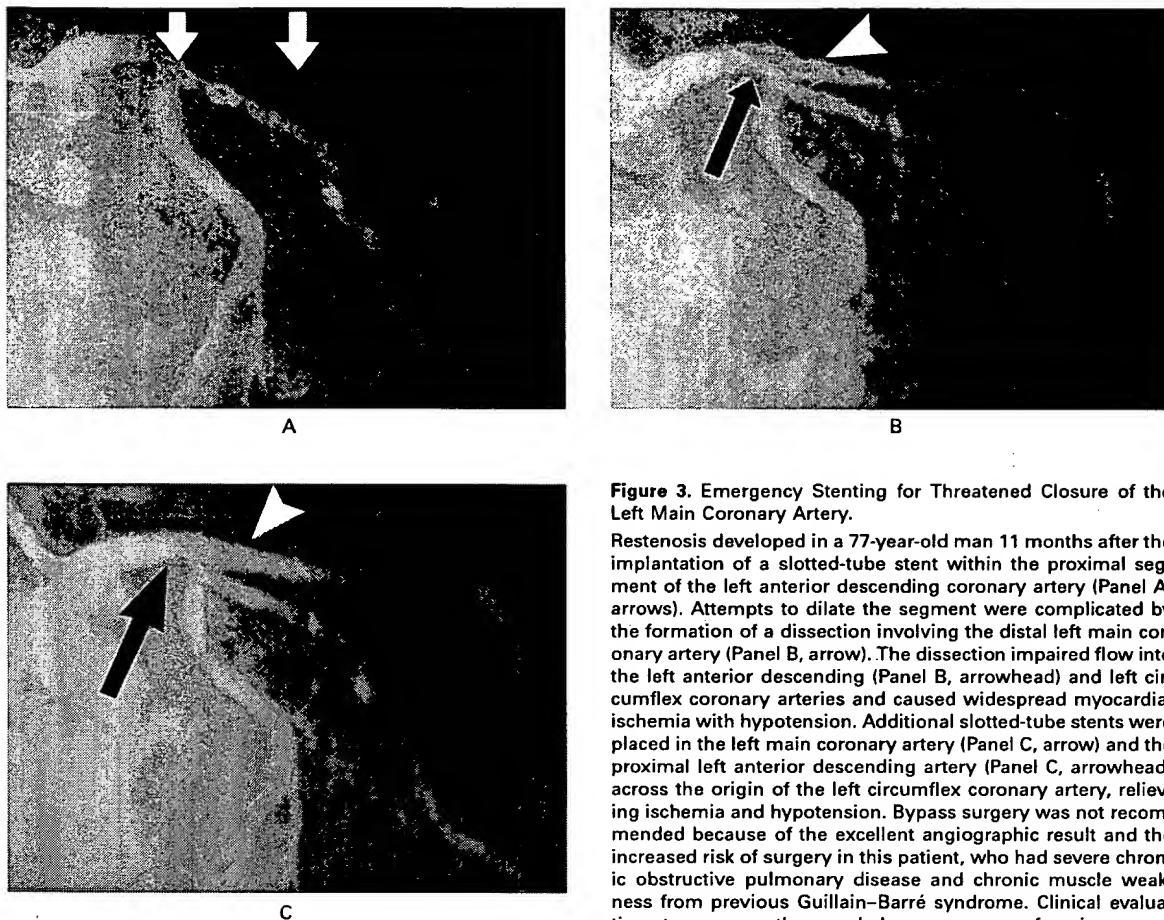


Figure 3. Emergency Stenting for Threatened Closure of the Left Main Coronary Artery.

Restenosis developed in a 77-year-old man 11 months after the implantation of a slotted-tube stent within the proximal segment of the left anterior descending coronary artery (Panel A, arrows). Attempts to dilate the segment were complicated by the formation of a dissection involving the distal left main coronary artery (Panel B, arrow). The dissection impaired flow into the left anterior descending (Panel B, arrowhead) and left circumflex coronary arteries and caused widespread myocardial ischemia with hypotension. Additional slotted-tube stents were placed in the left main coronary artery (Panel C, arrow) and the proximal left anterior descending artery (Panel C, arrowhead) across the origin of the left circumflex coronary artery, relieving ischemia and hypotension. Bypass surgery was not recommended because of the excellent angiographic result and the increased risk of surgery in this patient, who had severe chronic obstructive pulmonary disease and chronic muscle weakness from previous Guillain-Barré syndrome. Clinical evaluation at seven months revealed no recurrence of angina.

ceive anticoagulation therapy; and more than two thirds of all patients did not undergo intravascular ultrasound imaging.⁴⁷

New information has recently appeared on antithrombotic therapy after stent implantation. Neumann and colleagues⁵⁰ reported that activation of platelets, rather than the coagulation pathway, increases the risk of stent-associated thrombosis. This observation was the basis for a randomized trial comparing the combination of ticlopidine and aspirin with the anticoagulant phenprocoumon and aspirin. The former regimen resulted in lower rates of stent-associated thrombosis (0.8 percent vs. 5.4 percent, $P = 0.004$) and major hemorrhage (0.0 percent vs. 6.5 percent, $P < 0.001$).⁵¹

To date, no study has demonstrated that the use of stents made of bare stainless steel and anticoagulation therapy with warfarin results in a lower incidence of death or nonfatal myocardial infarction than does PTCA.^{5,6,32,33,39} Several randomized trials are under way to determine whether alternative antithrombotic approaches reduce the risk of major

complications of coronary-stent implantation as compared with PTCA.

Innovations in Coronary Stents

New stent designs, such as the heparin-coated stent evaluated in the Belgium–Netherlands Stent II pilot study, may have important clinical benefits.⁵² There were no instances of documented stent-associated thrombosis in 207 consecutive patients, among whom 2.0 percent died, 1.5 percent had myocardial infarction, 2.0 percent needed bypass surgery, 1.5 percent had stroke, and 8.9 percent underwent repeated angioplasty within seven months after stent implantation for stable angina. This finding suggests that use of the optimal design, implantation technique, and antithrombotic therapy in selected patients could reduce the rate of subacute thrombotic occlusion seen with bare metallic stents and anticoagulation with warfarin (Table 2).

The stents currently approved for use in the United States are the slotted-tube and flexible-coil designs, both made of bare stainless steel (Fig. 2).

Eighteen new stent designs are under investigation in Europe and North America, including welded tubular stents, integrated flexible-coil stents, self-expanding stents, interlocking coil-strut stents, and radiation-emitting stents. It is unlikely that a single design will be suitable for all patients, but the composition and structure of the stent are likely to have important clinical consequences. For example, an experimental study found lower rates of thrombosis and lumen narrowing with the use of a stainless-steel, corrugated-ring stent than with a stainless-steel, slotted-tube stent of identical mass and diameter.⁵³

Coronary Stenting and the Process of Restenosis

New concepts of restenosis have emerged simultaneously with the widespread use of coronary stents. Early studies suggested that intimal proliferation is the predominant cause of narrowing of the lumen after arterial injury in animals with normal or elevated cholesterol levels. On the basis of the results of experimental studies, more than 50 trials enrolling more than 13,000 patients have evaluated various drugs to block intimal proliferation after coronary angioplasty, but none of the drugs have produced consistently beneficial results.⁵⁴

Recent studies have challenged the proliferation model of restenosis. Using immunohistochemical labeling of proliferative-cell nuclear antigen in restenotic lesions extracted with directional atherectomy, O'Brien and colleagues⁵⁵ found only scanty evidence of cellular proliferation and no obvious temporal peak during a six-month period after angioplasty. Mintz and colleagues^{56,57} used serial intravascular ultrasound imaging after various coronary interventions to quantify the separate contributions of vessel-wall geometry, atherosclerotic plaque, and the vessel lumen to the process of restenosis. They found that intimal thickening accounted for about 30 percent of the loss in lumen diameter six months after coronary interventions, whereas shrinkage of the dilated segment, measured as a reduction in the cross-sectional area of the vessel subtended by the external elastic lamina, accounted for most of the loss.^{56,57}

Current studies thus suggest that restenosis is predominantly influenced by arterial remodeling — a process that consists of either an adaptive increase or a pathologic shrinkage of the cross-sectional area of the vessel (Fig. 4). Whereas adaptive remodeling has been identified in the course of human coronary atherosclerosis as a mechanism that delays the development of focal stenoses in the presence of enlarging atheromas,⁵⁸ pathologic remodeling has been observed as a process that increases the encroachment of atheroma and neointima on the arterial lumen, as was observed initially in studies of restenosis in rabbits.^{59,60}

Coronary stenting reduces the incidence of restenosis because it produces large lumens¹⁵ and staves

off pathologic remodeling.⁶¹ Serial intravascular ultrasound studies suggest that neointimal proliferation through the stent struts accounts for almost all the late loss in lumen diameter after coronary stenting, with almost no evidence of vessel shrinkage or collapse of the stent (Fig. 4).⁶¹

NEW ANTITHROMBOTIC THERAPIES FOR USE DURING CORONARY ANGIOPLASTY

In 1994 the mainstay of anticoagulation therapy during PTCA was the combination of aspirin (325 mg daily) and heparin in a dose sufficient to achieve an activated clotting time of more than 300 seconds during the procedure.^{13,46} Since 1994, several new antithrombotic therapies for PTCA have been tested.

The extent of arterial-thrombus formation during coronary angioplasty depends on the degree of platelet activation on exposure to thrombogenic components of atheromatous plaques⁶² and changes in shear caused by stenoses.⁶³ Thrombin is generated during coronary angioplasty⁶⁴ and potentially activates platelets.⁶⁵ Because heparin has several limitations as a thrombin inhibitor, including its requirement for co-factor antithrombin III and inhibition within platelet-rich thrombi,⁶⁶ direct thrombin inhibitors have been developed as possible substitutes. Two multicenter studies^{67,68} evaluated the direct thrombin inhibitors hirudin and bivalirudin in patients undergoing PTCA for unstable angina and found that these agents were marginally better than heparin in preventing ischemic complications. The failure of direct thrombin inhibitors to show a striking advantage over heparin during angioplasty is now attributed to the multiplicity of pathways for platelet activation⁶⁹ and the inability of these agents, unlike heparin, to block the generation of thrombin.⁶⁷

Whereas there are multiple pathways for platelet activation, a single receptor mediates the process of platelet aggregation. The platelet glycoprotein IIb/IIIa receptor binds fibrinogen to cross-link platelets but is blocked irreversibly by the monoclonal antibody abciximab, or c7E3. In the Evaluation of Abciximab for the Prevention of Ischemic Complications (EPIC) study,¹⁰ 2099 patients undergoing PTCA or directional atherectomy for acute myocardial infarction, refractory unstable angina, or high-risk coronary stenoses were treated with heparin and aspirin and randomly assigned to additional treatment with placebo, a bolus of abciximab, or a bolus followed by an infusion of abciximab. Treatment with abciximab as a bolus and infusion significantly reduced the combined end point of death, myocardial infarction, or repeated revascularization at 30 days (Table 3). At six months, patients treated with abciximab as a bolus and infusion had a significantly lower incidence of major ischemic complications or repeated revascularization than those given placebo.¹¹ These favorable long-term effects were

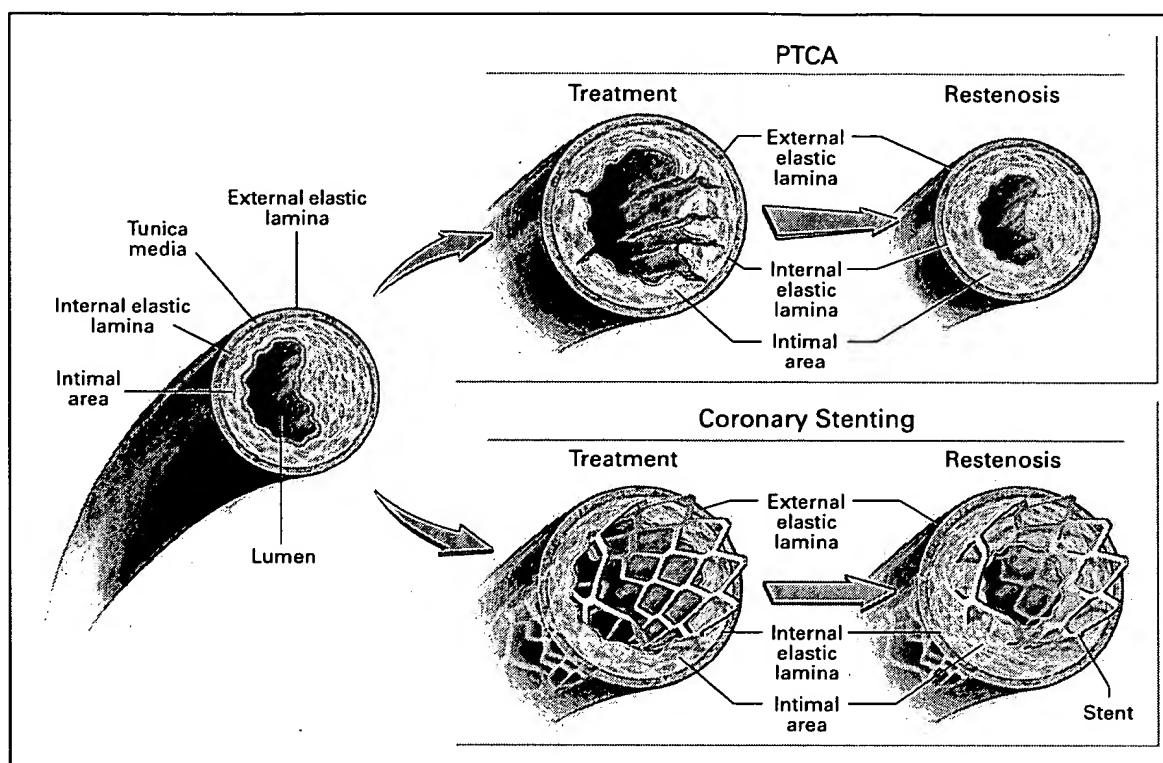


Figure 4. Possible Mechanisms of Restenosis after PTCA and Coronary Stenting.

Serial intravascular ultrasound studies suggest that PTCA almost always disrupts plaque without reducing the total intimal area, frequently causes dissections that penetrate into the tunica media through the internal elastic lamina, and transiently enlarges the vessel, measured as the cross-sectional area subtended by the external elastic lamina. Restenosis is caused by pathologic arterial remodeling, characterized by shrinkage of the area circumscribed by the external elastic lamina, and to a lesser extent by neointimal thickening. Coronary stenting also enlarges the cross-sectional area of the vessel. The radial force of the stent prevents vessel shrinkage, but neointimal proliferation can be excessive.

predominantly due to a reduced need for bypass surgery or repeated angioplasty, results consistent with a reduction in the incidence of clinical restenosis.

The benefits of abciximab in the EPIC study were counterbalanced by increased rates of major hemorrhage or the need for transfusion (7.0, 13.2, and 15.4 percent in the groups that received placebo, abciximab as a bolus, and abciximab as a bolus and infusion, respectively).¹⁰ Additional studies, however, optimized the safety of abciximab during PTCA by combining it with aspirin and lower doses of heparin adjusted to body weight. In the study entitled "Evaluation of PTCA to Improve Long-term Outcomes with c7E3 Glycoprotein IIb/IIIa-Receptor Blockade (EPILOG)," Topol and colleagues used abciximab with low-dose heparin (70 units per kilogram of body weight) or standard-dose heparin (100 units per kilogram) and observed rates of major hemorrhage (2.0 percent and 3.5 percent, respectively) that were substantially lower than those seen in the EPIC study¹² (and Topol EJ: personal communication). The use of weight-adjusted hepa-

rin also preserved the clinical efficacy of abciximab. The incidence of the combined end point of death or nonfatal myocardial infarction at 30 days was 3.8 percent for patients treated with abciximab and low-dose heparin, 4.2 percent for those treated with abciximab and standard-dose heparin, and 9.1 percent for those treated with standard-dose heparin alone ($P < 0.001$) (Topol EJ: personal communication).

Indications for abciximab therapy during coronary angioplasty are evolving. Although large clinical studies such as EPIC and EPILOG prove that blockade of platelet glycoprotein IIb/IIIa receptors is useful during angioplasty, these studies do not define the details of optimal patient selection. The greatest treatment benefit of abciximab appears to be in patients with unstable angina refractory to anticoagulation with heparin, acute myocardial infarction, or postinfarction angina. Abciximab is also useful during angioplasty when unstable angina is associated with angiographic evidence of thrombus. Other indications, such as pretreatment for patients

TABLE 3. EFFECTIVENESS OF NEW ANTITHROMBOTIC APPROACHES IN CORONARY ANGIOPLASTY.*

END POINT	EPIC STUDY†			IMPACT II STUDY‡		
	PLACEBO (N = 696)	ABCIXIMAB BOLUS (N = 695)	ABCIXIMAB BOLUS AND INFUSION (N = 708)	PLACEBO (N = 1285)	LOW-DOSE INTEGRILIN (N = 1286)	HIGH-DOSE INTEGRILIN (N = 1300)
	percentage of patients					
Events at 30 days						
Death	1.7	1.3	1.7	1.1	0.5	0.9
Nonfatal MI	8.6	6.2	5.2§	7.5	6.4	6.5
Emergency PTCA	4.5	3.6	0.8§	1.0	1.1	1.1
Emergency CABG	3.6	2.3	2.4	1.4	0.8	1.2
Death, MI, or revascularization	—	—	—	—	—	—
Events at 6 mo						
Death	3.4	2.6	3.1	—	—	—
MI	10.5	8.0	6.9§	—	—	—
PTCA	20.9	19.9	14.4	—	—	—
CABG	10.9	9.9	9.4	—	—	—
Any event	35.1	32.6	27.0§	—	—	—

*EPIC denotes Evaluation of 7E3 for the Prevention of Ischemic Complications, IMPACT Integrilin to Minimize Platelet Aggregation and Prevent Coronary Thrombosis, MI myocardial infarction, and CABG coronary-artery bypass grafting.

†Data were obtained from the EPIC investigators.¹⁰ Patients with acute myocardial infarction, unstable angina, or high-risk lesions were studied.

‡Data were obtained from Tchong et al.⁷⁰ and Horrigan et al.⁷¹ Patients with unstable or stable angina were studied. Of the 4010 randomized patients, 3871 were treated with either placebo or Integrilin.

§P<0.05 for the comparison with heparin or placebo.

with unstable angina awaiting coronary angioplasty, are currently being evaluated in clinical trials.

Reversible, non-antibody-based blockers of platelet glycoprotein IIb/IIIa receptor have also been evaluated during angioplasty in multicenter, randomized clinical trials (Table 3). In the study entitled "Integrilin to Minimize Platelet Aggregation and Prevent Coronary Thrombosis II,"⁷⁰ which involved 4010 patients undergoing angioplasty or atherectomy, high doses of the cyclic heptapeptide Integrilin were associated with a lower incidence of major ischemic complications or emergency revascularization than placebo at 24 hours (9.3 percent vs. 7.0 percent, $P=0.03$), but no treatment effect was apparent at 30 days (11.4 percent vs. 9.9 percent, $P=0.22$).⁷¹ The differences between the treatment effects of abciximab and those of Integrilin may relate to the irreversibility of action of abciximab or its cross-reactivity with other vascular receptors.

Thrombolytic Therapy as Adjunctive Therapy

Thrombolytic agents have been evaluated in clinical trials of PTCA. In the Thrombolysis and Angioplasty in Unstable Angina Trial,⁷² 469 patients with angina at rest who were undergoing PTCA were treated with heparin and aspirin and randomly assigned to receive placebo or intracoronary uroki-

nase. Urokinase resulted in a lower incidence of angiographic evidence of thrombus during angioplasty (13.8 percent vs. 18.0 percent), but in higher rates of abrupt vessel closure (10.2 percent vs. 4.3 percent, $P<0.02$) and the combined end point of recurrent ischemia, myocardial infarction, or emergency bypass surgery (12.9 percent vs. 6.3 percent, $P<0.02$).⁷² In the Thrombolysis in Myocardial Ischemia (TIMI) IIIB study,⁷³ 1473 patients with unstable angina or non-Q-wave myocardial infarctions were treated with heparin and randomly assigned to receive adjunctive tissue plasminogen activator or placebo. At 42 days the rate of myocardial infarction was higher in the group treated with tissue plasminogen activator (7.4 percent vs. 4.9 percent, $P=0.04$).⁷³

These two studies suggest that thrombolytic therapy should not be used routinely during angioplasty for unstable angina. The deleterious effects of thrombolytic therapy in this setting may be caused by the platelet-activating actions of thrombolytic agents.⁷⁴

COMPARISON OF ANGIOPLASTY WITH OTHER THERAPIES

Choosing among medical therapy, angioplasty, and surgical treatments remains a difficult decision in the care of individual patients with coronary ar-

TABLE 4. COMPARISON OF MEDICAL THERAPY AND CORONARY ANGIOPLASTY IN PATIENTS WITH STABLE ANGINA, UNSTABLE ANGINA, OR NON-Q-WAVE MYOCARDIAL INFARCTION.*

END POINT	ACME Study†		MASS‡		LITA	TIMI IIIB Study§	
	MEDICAL THERAPY (N=107)	PTCA (N=105)	MEDICAL THERAPY (N=72)	PTCA (N=72)	SURGICAL GRAFT (N=70)	CONSERVATIVE THERAPY (N=733)	INVASIVE THERAPY (N=740)
	percentage of patients						
Death	0.9	0.0	0.0	1.4	1.4	4.4	4.1
MI	2.8	4.8	2.8	2.8	1.4	8.3	9.3
CABG	0.0	6.7¶	5.6	13.9¶	100.0	30.0	30.0
Angina	53.9	36.5¶	68.1	19.4¶	2.9¶	35.4	36.3
Repeated hospitalization	—	—	—	—	—	32.5	25.8¶

*ACME denotes Angioplasty Compared with Medicine, MASS Medicine, Angioplasty or Surgery Study, TIMI Thrombolysis in Myocardial Ischemia, LITA left internal thoracic artery, MI myocardial infarction, and CABG coronary-artery bypass grafting.

†Data were obtained from Parisi et al.⁷⁵ Patients with stable angina and single-vessel coronary artery disease were studied. The results reported are for the six-month follow-up.

‡Data were obtained from Hueb et al.⁷⁶ Patients with stable angina and a single stenosis in the left anterior descending coronary artery were studied. The results reported are for the three-year follow-up.

§Data were obtained from Anderson et al.⁷⁷ Patients with unstable angina or non-Q-wave myocardial infarction were studied. Conservative therapy involved admission to the hospital and immediate treatment with anti-ischemic medications, heparin, and aspirin. Invasive therapy involved cardiac catheterization within 18 to 24 hours and coronary angioplasty or bypass surgery at the discretion of the investigator. The results reported are for the one-year follow-up.

¶P<0.05.

tery disease. Nonetheless, the results of several clinical trials allow general guidelines to be developed.

PTCA versus Medical Therapy

PTCA has been compared with medical therapy for stable angina in two studies. In the Angioplasty Compared with Medicine study,⁷⁵ patients with stable angina and single-vessel coronary disease were randomly assigned to treatment with PTCA or medical therapy. In the Medicine, Angioplasty, or Surgery Study,⁷⁶ patients with stable angina and a stenosis in the proximal left anterior descending artery were randomly assigned to medical therapy, PTCA, or bypass surgery with the left internal thoracic artery (Table 4). Both studies suggested that PTCA provides more complete relief of angina than medical therapy but is associated with higher rates of myocardial infarction or bypass surgery (Table 4).

The TIMI IIIB study^{73,77} addressed the benefit of PTCA for patients with unstable angina or non-Q-wave myocardial infarction. An aggressive strategy of early cardiac catheterization with angioplasty was compared with a conservative strategy of medical therapy for patients presenting with ischemic pain at rest (Table 4). Although major complications occurred with similar frequencies in patients assigned to the two groups, more post-discharge procedures

and hospitalizations were required by the patients assigned to the conservative strategy, suggesting that PTCA provides more rapid and complete relief of angina without increasing the risk of major complications.^{73,77}

Several studies have evaluated PTCA in patients with acute myocardial infarction (Table 5). A meta-analysis of several reports suggested that PTCA performed without antecedent thrombolytic therapy results in a lower risk of death or reinfarction than thrombolytic therapy.⁷⁸ Whether PTCA can be used successfully in a broad range of settings for acute myocardial infarction remains unclear. An analysis of results from the Myocardial Infarction Triage and Intervention Registry showed nearly identical hospital mortality rates for direct angioplasty and thrombolytic therapy for acute myocardial infarction in 19 Seattle hospitals (5.5 percent vs. 5.6 percent).⁷⁹

Thus, the results of clinical trials comparing PTCA with medical therapy suggest that the benefit of angioplasty depends on the severity and acuity of the clinical presentation. A gradient of risk extends across the spectrum of patients with coronary artery disease treated medically (Tables 4 and 5). At one end of the spectrum, patients with stable angina and mild coronary artery disease treated medically are at low risk of death or nonfatal myocardial infarction.

TABLE 5. COMPARISON OF MEDICAL THERAPY AND CORONARY ANGIOPLASTY IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION (MI).

END POINT	MICHELIS AND YUSUF*	
	PTCA (N = 571)	THROMBOLYSIS (N = 574)
	percentage of patients	
Death	4	6†
Death or repeated MI	6	11†

*This study of results at six weeks was a meta-analysis.⁷⁸

†P<0.05.

In this setting, PTCA effectively relieves angina, with a low risk of complications, but does not lower the risk of death, myocardial infarction, or future revascularization procedures. At the other end of the spectrum, patients with acute myocardial infarction treated with thrombolytic therapy have a risk of major complications such as reinfarction or stroke that may be reduced with early angioplasty. In the middle of the spectrum, patients with unstable angina treated medically have an intermediate risk of major ischemic complications. In this setting, PTCA provides symptomatic relief and stabilizes the course of unstable angina without increasing the risk of death or nonfatal myocardial infarction.

PTCA versus Bypass Surgery

Several studies have compared PTCA with bypass surgery for patients with single-vessel or multivessel coronary artery disease. Despite the use of different

protocols, these studies have yielded consistent results (Table 6).^{76,80-84} Major ischemic complications, such as death or myocardial infarction, occur with similar frequencies one to five years after angioplasty or bypass surgery. The chief difference between the two strategies is the increased need for repeated revascularization procedures in patients who initially underwent PTCA. In the Bypass Angioplasty Revascularization Investigation (BARI), however, 69 percent of patients initially treated with angioplasty had not undergone bypass surgery five years later.⁸¹

Thus, patients with single-vessel or multivessel coronary artery disease who are good candidates for either angioplasty or bypass surgery can be reassured that both revascularization approaches are followed by equivalent rates of major ischemic complications. However, diabetic patients had higher rates of restenosis than patients without diabetes⁸⁵ and significantly higher rates of survival five years after treatment with bypass surgery than with coronary angioplasty in BARI (81 percent vs. 65 percent, P=0.003).⁸¹ In the absence of other factors affecting surgical risk, the decision about surgical therapy or angioplasty can be based on personal preference, weighing the invasive nature of bypass surgery against the likelihood of repeated procedures after angioplasty.

Relation between Severity of Stenosis and Clinical Outcome

Why have coronary interventional therapies not improved the natural history of coronary artery disease more substantially? Although it seems intuitive that the risk of complications from coronary atherosclerosis should correlate with the angiographic se-

TABLE 6. COMPARISON OF SURGICAL THERAPY AND CORONARY ANGIOPLASTY.*

END POINT	POCOCK ET AL.†		POCOCK ET AL.‡		BARI STUDY§	
	CABG (N = 358)	PTCA (N = 374)	CABG (N = 1303)	PTCA (N = 1336)	CABG (N = 914)	PTCA (N = 915)
	percentage of patients					
Death	0.3	1.9	2.8	3.1	10.7	13.7
Death or MI	4.5	7.2	8.5	8.1	11.7	10.9
Repeated CABG	1.4	16.0¶	0.8	18.3¶	0.7	20.5¶
Repeated CABG or PTCA	3.6	30.5¶	3.2	34.5¶	8.0	54.0¶
More than mild angina	6.5	14.6¶	12.1	17.8¶	—	—

*BARI denotes Bypass Angioplasty Revascularization Investigation, CABG coronary-artery bypass grafting, and MI myocardial infarction.

†This study⁸⁰ was a meta-analysis of the results of three trials at one year. Patients with single-vessel disease were studied.

‡This study⁸⁰ was a meta-analysis of the results of six trials at one year. Patients with multivessel disease were studied.

§Data were obtained from the BARI investigators.⁸¹ Patients with multivessel disease were studied. The results reported are for the five-year follow-up.

¶P<0.05.

verity of individual coronary-artery lesions, several studies have discounted this notion.

Angiographic studies of cholesterol reduction have defined the relation between the severity of lesions and clinical events. In the Familial Atherosclerosis Treatment Study,⁸⁶ men with coronary artery disease were randomly assigned to treatment with placebo or one of two lipid-lowering regimens, lovastatin plus colestipol or niacin plus colestipol. After two years of treatment, there was a striking reduction in the rate of clinical events despite a slight 0.3 and 1.1 percentage-point reduction in the severity of stenosis in the two groups receiving active treatment. Myocardial infarction occurred in 19 percent of the control group, 4 percent of the group receiving lovastatin plus colestipol, and 6 percent of the group receiving niacin plus colestipol.⁸⁶

Although lipid-lowering therapy does not reduce the likelihood of restenosis six months after coronary angioplasty,⁸⁷ it does reduce the likelihood of angina or myocardial infarction and the need for revascularization procedures in patients with hypercholesterolemia. Intensive lipid-lowering therapy was associated with a 34 percent reduction in major ischemic events and a 37 percent reduction in the rate of bypass surgery or PTCA in the Scandinavian Simvastatin Survival Study⁸⁸ and similar reductions in the Cholesterol and Recurrent Events Study.⁸⁹ The mechanism for this benefit has been attributed to minor regression of fixed stenoses, generalized improvement in endothelial function, and a decreased risk of plaque rupture during lipid-lowering therapy.^{90,91}

The dissociation between the severity of stenosis and the risk of major ischemic complications has been confirmed in several other settings. Ambrose and colleagues⁹² compared coronary stenoses in 38 patients who had myocardial infarction in the interval between two coronary arteriographic studies. The lesions seen on the initial angiogram that were later responsible for Q-wave myocardial infarctions had a mean stenosis of only 34 percent. In a similar study, Little and colleagues⁹³ reviewed serial coronary arteriograms in 42 consecutive patients before and after acute myocardial infarction. In 29 patients, a new total occlusion was observed on the second arteriogram. Among these 29 patients, 66 percent of the culprit lesions were associated with stenoses of less than 50 percent on the initial arteriogram.

Thus, the emphasis on the severity of stenosis wrongly reinforces the simplistic notion that angiographically severe lesions are associated with an increased risk of death or myocardial infarction. Whereas coronary angioplasty is appropriate to reduce symptoms in patients with angina and angiographically severe lesions, because these are frequently associated with decreased coronary flow

reserve and myocardial ischemia,⁹⁴ this therapy cannot be expected to eliminate all subsequent cardiovascular risk, because coronary atherosclerosis is a diffuse process⁹⁵ and angiographically mild stenoses not conventionally targeted for treatment are inherently more likely than severe lesions to cause myocardial infarctions.^{92,93}

Although coronary arteriography provides a high-resolution image of the coronary lumen, it may not be ideal for evaluating the severity of coronary atherosclerosis — a disease of the vessel wall. Additional information about coronary atherosclerosis may be obtained with new imaging techniques and physiologic measurements. Intravascular ultrasound imaging provides anatomical details of the vessel wall and atherosclerotic plaque that are helpful in some coronary interventions.⁹⁶ Several studies are under way to determine whether ultrasound findings are associated with clinical outcomes.⁹⁷

CONCLUSIONS

PTCA has been a major therapeutic advance because it relieves angina in patients with severe stenoses. Coronary stenting has revolutionized the practice of interventional cardiology by partially overcoming some of the limitations of coronary angioplasty, such as abrupt vessel closure and restenosis. Ongoing studies of stent coatings and optimal antithrombotic therapies may improve the success of coronary-stent implantation, further reduce the rate of restenosis, and lower the risk of death or nonfatal myocardial infarction.

Platelet glycoprotein IIb/IIIa-receptor blockers have reduced the rate of acute complications of coronary angioplasty in high-risk settings and may have persistent benefits after discharge from the hospital. Further studies are under way to confirm the potential long-term benefits and to evaluate the role of these agents as treatment for unstable angina or myocardial infarction before coronary angioplasty is performed.

In general, the treatment effect of mechanical coronary interventions is confined to discrete coronary-artery segments, whereas the pathologic process of coronary atherosclerosis is diffuse. Coronary interventional therapy should thus be viewed as part of a comprehensive strategy involving other treatments, such as intensive efforts to lower lipid levels, which may halt the generalized progression of disease and reduce the risk of death or myocardial infarction.

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REVIEW ARTICLES

MECHANISMS OF DISEASE

FRANKLIN H. EPSTEIN, M.D., *Editor*PLATELET GLYCOPROTEIN IIb/IIIa
RECEPTORS IN CARDIOVASCULAR
MEDICINEJEFFREY LEFKOVITS, M.B., B.S.,
EDWARD F. PLOW, PH.D.,
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Platelets have a key role in atherosclerosis, thrombosis, and acute coronary syndromes. Therapeutic manipulation of platelet function has focused principally on the use of aspirin, which has proved effective in many clinical situations, despite its relatively weak antiplatelet action. More recently, the platelet glycoprotein IIb/IIIa receptor has been identified as the pivotal mediator of platelet aggregation, making it a logical target for control of the platelet response to vascular injury. This review summarizes the current state of knowledge of the biology of glycoprotein IIb/IIIa receptors and examines the development of agents that inhibit these receptors — a diverse group of drugs specifically designed to alter platelet function.

BIOLOGY OF PLATELET FUNCTION

Platelet Adhesion and Aggregation

Platelet adhesion, the first step in the process of hemostasis, is triggered by damage to the vessel wall and local exposure of the subendothelial matrix. Coverage of the exposed site by platelets depends on the recognition of adhesive proteins by specific platelet-membrane glycoproteins, many of which are integrins (reviewed by Hynes¹ and Smyth et al.²). The integrin family consists of heterodimeric molecules composed of a series of α and β subunits. Specific combinations of these subunits form receptors with unique specificities for various ligands. Integrins are found on virtually all cells and mediate many different physiologic responses. The major integrins present on the surface of platelets — all of which have been shown to play a part in the process of platelet adhesion — are listed in Table 1.

The glycoprotein Ib receptor (a nonintegrin), which exists in a complex with glycoprotein IX and glycopro-

tein V on the platelet surface, binds von Willebrand factor and is the principal glycoprotein involved in the initial contact between platelets and the vessel wall.^{3,4} The glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) integrin, in addition to its function in platelet aggregation, has a secondary role in platelet adhesion.^{5,6} The glycoprotein Ia/IIa ($\alpha_2\beta_1$) integrin appears to be a principal platelet receptor for collagen,^{7,8} and glycoprotein IV, a nonintegrin, may also play a part in platelet-collagen interactions, as well as acting as a receptor for thrombospondin.^{9,10} Other integrins that contribute to platelet adhesion include glycoprotein Ic/IIa ($\alpha_3\beta_1$), a fibronectin receptor; $\alpha_6\beta_1$, a laminin receptor; and $\alpha_v\beta_3$, a vitronectin receptor that also recognizes many of the ligands that bind to the glycoprotein IIb/IIIa receptor.¹¹

Platelet activation follows adhesion and can be initiated by several mechanical and chemical stimuli. Adhesion of platelets to collagen and other components of the subendothelial matrix and the presence of thrombin are among the strongest stimulators of platelet activation. The activation of platelets is associated with stimulation of several metabolic pathways, changes in the shape of platelets, activation of the glycoprotein IIb/IIIa receptor, and induction of platelet coagulant activity.¹² Among the substances that stimulate these intracellular pathways are thromboxane A_2 , thrombin, norepinephrine, collagen, and adenosine diphosphate. They act through various receptors and secondary messengers, such as diacylglycerol and inositol triphosphate,¹³ to stimulate intracellular mobilization of calcium and degranulation of platelets. Platelet release of

Table 1. Platelet-Membrane Glycoprotein Receptors Involved in the Adhesion and Aggregation of Platelets.

RECEPTOR	LIGAND	RECEPTOR-MEDIATED ACTION	AMINO ACID SEQUENCE RECOGNIZED
Integrin			
$\alpha_2\beta_1$ (glycoprotein Ia/IIa)	Collagen	Adhesion	DGEA*
$\alpha_5\beta_1$ (glycoprotein Ic/IIa)	Fibronectin	Adhesion	RGD
$\alpha_6\beta_1$	Laminin	Adhesion	Not confined to a short sequence
$\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa)	Fibrinogen	Aggregation	KQAGDV or RGD
	Fibronectin		RGD*
	von Willebrand factor		RGD
	Vitronectin		RGD
$\alpha_v\beta_3$	Vitronectin	Adhesion	RGD
	Fibrinogen		RGD
	Fibronectin		RGD
	von Willebrand factor		RGD
Nonintegrin			
Glycoprotein Ib	von Willebrand factor	Adhesion	Not confined to a short sequence
Glycoprotein IV	Thrombospondin	Adhesion	CSVTCG
	Collagen		?

*Other amino acid sequences may also be involved.

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adenosine diphosphate, serotonin, and thromboxane A_2 , together with the presence of other agonists in the microenvironment, triggers the recruitment and activation of surrounding platelets.¹⁴

Irrespective of the agonist, the final common pathway leading to the formation of the platelet plug is platelet aggregation. This event is mediated by the binding of adhesive proteins to the now ligand-receptive form of the glycoprotein IIb/IIIa receptor (Fig. 1). Fibrinogen and von Willebrand factor are the principal adhesive macromolecules that link the platelets together by binding to glycoprotein IIb/IIIa molecules on adjacent platelets. As a result of multiple reactions of this type, the platelets become aggregated into a hemostatic plug.

Structure and Function of the Glycoprotein IIb/IIIa Receptor

The glycoprotein IIb/IIIa receptor ($\alpha_{IIb}\beta_3$) is a typical integrin (Fig. 2). Its 136-kd α subunit consists of a heavy chain and a light chain. The light chain has a short cytoplasmic tail, a transmembrane region, and a short extracellular domain, whereas the heavy chain is entirely extracellular.¹⁶ The 92-kd β subunit consists of a single polypeptide of 762 amino acids, with a short cytoplasmic tail, a single transmembrane region, and a large extracellular domain.^{17,18} Of the eight integrin β subunits, the α_{IIb} subunit has been found only in combination with β_3 , mostly in cells of the megakaryocyte lineage. The subunits are noncovalently bound to each other, and calcium is required to maintain the heterodimeric structure.^{19,20} The glycoprotein IIb/IIIa receptor is the most abundant integrin on the platelet surface, with approximately 50,000 copies per cell.

Early studies in patients with Glanzmann's thrombasthenia helped elucidate the role of the glycoprotein

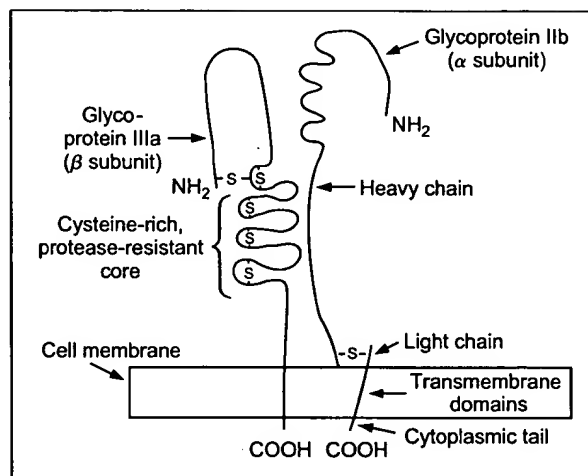


Figure 2. Diagram of the Platelet Glycoprotein IIb/IIIa Receptor. NH_2 denotes the amino group, $COOH$ the carboxyl group, and S the disulfide group. Adapted from Plow et al. with the permission of the publisher.¹⁵

IIb/IIIa receptor in platelet aggregation.^{21,22} This inherited disease, characterized by recurrent mucocutaneous bleeding but almost never by visceral bleeding, arises from genetic defects that result in an absence of or large decrease in functional glycoprotein IIb/IIIa receptors.²³ The disease is associated with deficient platelet aggregation in response to all physiologic stimuli and provides a paradigm for the pharmacologic inhibition of glycoprotein IIb/IIIa receptors.

Studies of patients with Glanzmann's thrombasthenia also demonstrated that the glycoprotein IIb/IIIa receptors bind a number of adhesive proteins.²⁴⁻²⁶

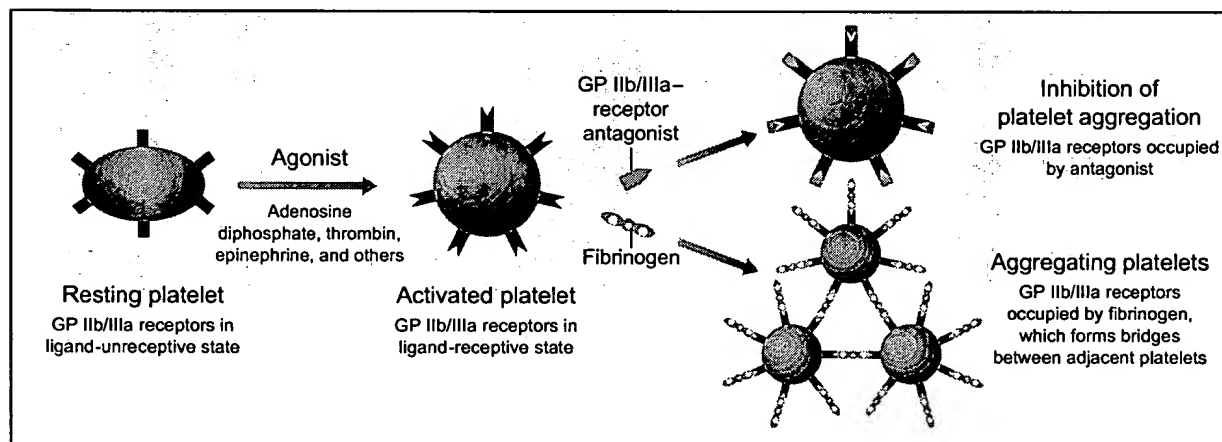


Figure 1. Overview of the Processes of Platelet Activation and Aggregation and the Inhibition of Platelet Aggregation by Inhibitors of Glycoprotein (GP) IIb/IIIa Receptors.

Platelet activation causes changes in the shape of platelets and conformational changes in glycoprotein IIb/IIIa receptors, transforming the receptors from a ligand-unreceptive to a ligand-receptive state. Ligand-receptive glycoprotein IIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets and facilitate platelet aggregation. Inhibitors of glycoprotein IIb/IIIa receptors also bind to glycoprotein IIb/IIIa receptors, blocking the binding of fibrinogen and thus preventing platelet aggregation.

Although the binding of fibrinogen to glycoprotein IIb/IIIa receptors is the principal mechanism for platelet aggregation,²⁷⁻²⁹ other adhesive glycoproteins, including fibronectin, von Willebrand factor, and vitronectin, also bind to these receptors.^{30,31} The interactions between these ligands and the receptors are mutually exclusive.³² These other ligands appear to play a greater part in platelet adhesion to subendothelial structures mediated by glycoprotein IIb/IIIa receptors.¹⁸ In addition, interactions between von Willebrand factor and glycoprotein IIb/IIIa receptors may be important in platelet aggregation under conditions of high shear.³³

The recognition specificity of the glycoprotein IIb/IIIa receptor is defined by two peptide sequences. The Arg-Gly-Asp (RGD) sequence was initially identified as the adhesive sequence in fibronectin³⁴ but is also present in fibrinogen, von Willebrand factor, and vitronectin. All these ligands contain at least one RGD sequence, whereas fibrinogen contains two RGD sequences per half molecule. In fact, the RGD sequence is recognized by several integrins^{35,36} (Table 1). It is uncertain whether RGD itself is involved in the binding of fibrinogen to glycoprotein IIb/IIIa receptors or whether it mimics another binding site within fibrinogen. Some monoclonal antibodies to RGD do not disrupt platelet aggregation,³⁷ and recombinant fibrinogen lacking RGD still facilitates aggregation.³⁸ Nevertheless, peptides containing the RGD sequence are potent inhibitors of the interaction between glycoprotein IIb/IIIa receptors and fibrinogen,³⁹ and the introduction of RGD into nonadhesive proteins can confer platelet-adhesive properties on these proteins.⁴⁰

The other major sequence involved in the binding of fibrinogen to glycoprotein IIb/IIIa receptors is the Lys-Gln-Ala-Gly-Asp-Val sequence, located at the carboxyl terminus of the γ chain of fibrinogen.^{41,42} Unlike RGD, this sequence is found only in fibrinogen and is probably the predominant site for the binding of fibrinogen to glycoprotein IIb/IIIa receptors.^{38,43} The relation between this sequence and the RGD binding sites is not fully understood. γ -Chain peptides inhibit the binding of fibronectin and von Willebrand factor to platelets,³² possibly reflecting overlapping binding sites for RGD and γ -chain sequences on glycoprotein IIb/IIIa receptors or distinct sites that interact allosterically.^{15,44} These two sequences interact with several sites within the glycoprotein IIb/IIIa receptor. One hypothesis is that peptides containing RGD and those containing the γ chain have preferred but also shared contact sites, and these multiple sites contribute to the overall high-affinity binding of fibrinogen to glycoprotein IIb/IIIa receptors.¹⁵

In general, platelet activation is necessary for the binding of ligands, such as fibrinogen, to glycoprotein IIb/IIIa receptors. Platelet agonists trigger conformational changes in the receptor (inside-out signaling), making it receptive to the ligand. Ligand binding is

rapid while the plasma ligand concentrations are high and is followed by conformational changes in the ligand-receptor complex. Signals flow back to the platelet (outside-in signaling) to initiate such events as the assembly of a multiprotein cytoskeleton that is involved in reinforcement and contraction of the forming clot.^{45,46}

DEVELOPMENT OF GLYCOPROTEIN IIb/IIIa-RECEPTOR INHIBITORS

There is no better proof of the potential clinical benefit of platelet-inhibitor therapy than aspirin. Many trials have demonstrated the benefit of aspirin in patients with ischemic heart disease.^{47,48} Despite its efficacy, however, aspirin is a relatively weak antiplatelet drug, inhibiting only thromboxane A_2 -mediated platelet aggregation. Unlike aspirin, the new class of drugs that inhibit glycoprotein IIb/IIIa receptors prevents the binding of fibrinogen to these receptors, thereby inhibiting platelet aggregation, irrespective of the metabolic pathway responsible for initiating platelet aggregation (Fig. 1). As a group, these drugs have a wide range of potential uses, including primary prevention of cardiovascular disease and prevention of coronary events in patients at high risk.

Coller and his colleagues were the first to demonstrate that a murine monoclonal antibody directed against the glycoprotein IIb/IIIa receptor inhibited the binding of fibrinogen to platelets and thus inhibited platelet aggregation.⁴⁹ Subsequently, the Fc fragment of one such antibody, 7E3, was removed to prevent immunogenicity, and the Fab fragments were joined with the constant regions of human immunoglobulin, forming a chimeric compound (abciximab, or c7E3). This compound has undergone extensive clinical evaluation and was recently approved by the Food and Drug Administration for clinical use.

Natural products have also been screened extensively for activity against the glycoprotein IIb/IIIa receptor. Trigramin, isolated from the venom of the viper *Trimeresurus gramineus*, is a potent inhibitor of ligand binding to the glycoprotein IIb/IIIa receptor.⁵⁰ Its activity has been traced to an RGD sequence within a disulfide loop. Many RGD-containing peptides have since been isolated from the venom of several species of the viper family. These peptides are known as disintegrins.⁵¹⁻⁵⁴ As a group, however, they are likely to be antigenic and have therefore been used primarily as the basis for the design of synthetic low-molecular-weight antagonists.

Synthetic linear peptides based on the RGD template have relatively little activity and poor stability in plasma.⁵⁵ Cyclic RGD peptides are more resistant to enzymatic breakdown and theoretically have a higher potency.^{55,56} Agents in this group include G4120 (Genentech, South San Francisco, Calif.) and MK-852 (Merck, West Point, Pa.). Integrelin (COR Therapeutics, South San Francisco), a cyclic peptide that has al-

ready undergone extensive clinical evaluation, is based on a Lys/Gly/Asp sequence rather than an RGD sequence and may be a more specific inhibitor of glycoprotein IIb/IIIa receptors than RGD-containing peptides.⁵⁷

RGD derivatives can be modified in other ways to improve their activity and stability. Replacement of the arginine group in the RGD sequence with an amidino- or benzamidino-containing group and the use of D-amino acids increases the resistance of these compounds to enzymatic degradation.⁵⁵ Nonpeptide inhibitors of glycoprotein IIb/IIIa receptors do not have the α -amino acids of the peptide group (no peptide bonds), averting some of the problems associated with the peptide inhibitors, including a short survival time in the circulation. Drugs in these two groups include lamifiban (previously known as Ro 44-9883, Hoffmann-LaRoche, Basel, Switzerland) and tirofiban (previously called MK-383, Merck), both of which are now being studied in phase 3 clinical trials (Fig. 3).

Orally active inhibitors of glycoprotein IIb/IIIa receptors have also been developed. They have been de-

signed either as pro-drugs, which are metabolized to the active form after ingestion, or as inherently orally active agents.⁵⁵ In general, these two designs yield compounds with approximately equal potency, but the bioavailability of the pro-drugs tends to be greater. One orally active inhibitor of glycoprotein IIb/IIIa receptors, xemlofiban (previously called SC-54684, Searle, Skokie, Ill.) has undergone phase 1 and 2 testing.

Overall, these drugs are potent inhibitors of platelet aggregation. They cause a dose-dependent prolongation of skin template bleeding time and increase the risk of bleeding in various animal models of thrombosis.⁵⁸⁻⁶³ In phase 2 trials in humans, abciximab, Integrilin, lamifiban, and tirofiban, all of which must be given parenterally, have proved to be potent inhibitors of platelet aggregation with acceptable safety profiles.

CLINICAL STUDIES OF GLYCOPROTEIN IIb/IIIa-RECEPTOR INHIBITORS

The clinical evaluation of inhibitors of glycoprotein IIb/IIIa receptors has concentrated on the use of these agents in patients undergoing coronary angioplasty and

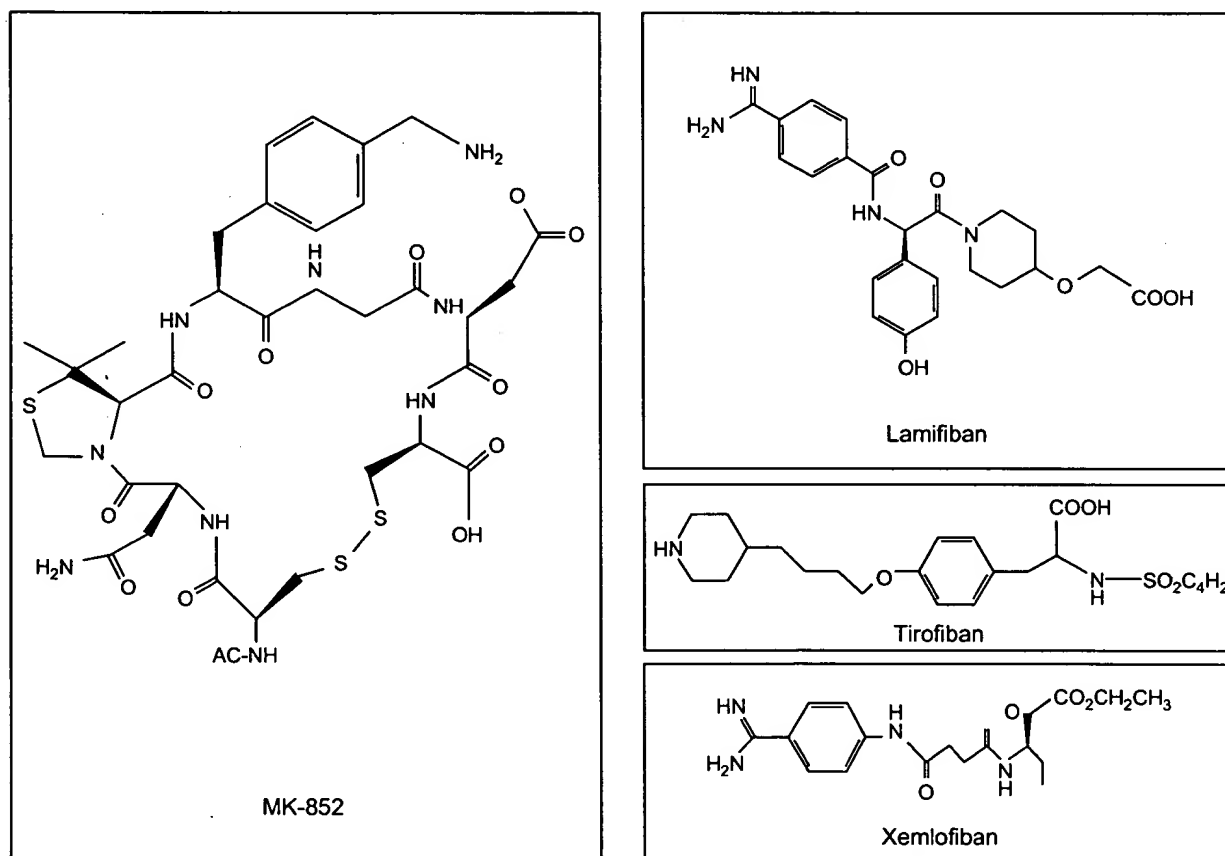


Figure 3. Chemical Structures of Some Inhibitors of Platelet Glycoprotein IIb/IIIa Receptors That Have Been Evaluated in Clinical Studies.

MK-852 is a cyclic RGD peptide, lamifiban is a peptide derivative, tirofiban is a nonpeptide, and xemlofiban is an orally active inhibitor of glycoprotein IIb/IIIa receptors. AC denotes acetyl group.

those with unstable angina and acute myocardial infarction.

Coronary Angioplasty

Early clinical studies demonstrated the ability of abciximab to produce a dose-dependent blockade of glycoprotein IIb/IIIa receptors during coronary angioplasty^{64,65} and to abolish cyclic variations in blood flow, a marker of platelet thrombus formation, in treated vessels after angioplasty.⁶⁶ These results provided the background for the large-scale Evaluation of c7E3 for the Prevention of Ischemic Complications (EPIC) trial.^{67,68} In this double-blind trial of 2099 patients undergoing coronary angioplasty or atherectomy, bolus administration of abciximab followed by a 12-hour infusion reduced the incidence of acute ischemic events by 35 percent, as compared with placebo. The principal effect of abciximab was to reduce the incidence of myocardial infarction.⁶⁹ However, the patients who received the drug had more bleeding events, mostly at sites of vascular access, than the patients who received placebo.

During the six-month follow-up period, the number of ischemic events (especially the need for a second revascularization) was reduced by 26 percent among the patients who received abciximab.⁶⁸ These results indicated that clinical restenosis could be decreased by a pharmacologic intervention. In support of this finding, experimental data have demonstrated that neointimal proliferation can be prevented by inhibition of platelet integrins.⁷⁰

Unstable Angina and Myocardial Infarction

Several inhibitors of glycoprotein IIb/IIIa receptors have been evaluated for use in patients with unstable angina. In the largest trial to date, therapy with lamifiban decreased the incidence of subsequent myocardial infarction and death in patients who had had chest pain during the preceding 24 hours.⁷¹ A similar benefit was shown in pilot studies of abciximab and Integrelin in patients with unstable angina.^{72,73} There are few data on the simultaneous administration of a thrombolytic agent and an inhibitor of glycoprotein IIb/IIIa receptors in patients with acute myocardial infarction, although the results of initial trials with abciximab and Integrelin are encouraging.^{74,75}

ISSUES THAT REMAIN TO BE ADDRESSED

The principal and most serious adverse effect of drugs that inhibit glycoprotein IIb/IIIa receptors is bleeding. In the EPIC trial, the rate of major bleeding was two times higher in the abciximab group than in the placebo group.⁶⁷ Patients who were older and those with lower body weight appeared to be at increased risk.⁷⁶ Bleeding may be minimized with lower doses of the drug and a shorter course of concomitant heparin therapy, together with early removal of the sheath after the procedure.⁷⁷ Nevertheless, the prevention of bleed-

ing will remain an important aim in the future clinical evaluation of these drugs.

The optimal duration of treatment with drugs that inhibit glycoprotein IIb/IIIa receptors has not been determined. In the EPIC trial, the patients who did not receive abciximab were at the greatest risk for abrupt closure of the treated coronary artery during the first two to three days after the angioplasty.⁶⁷ This finding, coupled with the effect noted at six months of follow-up, suggests the presence of blood-vessel passivation, in which the vessel wall has been converted from a platelet-reactive surface to a platelet-nonreactive surface. Blood-vessel passivation may require the inhibition of glycoprotein IIb/IIIa receptors for up to 72 hours or even longer. On the other hand, the development of orally active inhibitors of glycoprotein IIb/IIIa receptors will permit the study of the long-term effects of such treatment. Prolonged inhibition of glycoprotein IIb/IIIa receptors may expose epitopes that are normally induced only by the binding of adhesive ligands to receptors⁷⁸ or lead to the eventual loss of endothelial glycoprotein IIb/IIIa-related receptors. The consequences of these changes are not known.

The relative efficacy of the various inhibitors of glycoprotein IIb/IIIa receptors may also become an important issue. The monoclonal antibody abciximab stands out among these agents as relatively nonspecific, in that it binds to other integrins, including the vitronectin ($\alpha_v\beta_3$) receptor.⁷⁹ In contrast, several RGD derivatives appear to be specific for the platelet glycoprotein IIb/IIIa receptor and have little or no effect on other integrins. Whether these differences in specificity will have any influence on clinical efficacy is not known, but the absence of specificity for the glycoprotein IIb/IIIa receptor may prove to be clinically advantageous. Inhibition of the vitronectin receptor by abciximab may have contributed to the reduction in clinical restenosis among the patients who received this drug in the EPIC trial, in the same way that antagonism of the $\alpha_v\beta_3$ integrin by an RGD-peptide inhibitor reduced neointimal proliferation after balloon angioplasty in animals.⁸⁰

CONCLUSIONS

A growing understanding of the role of platelets in cardiovascular disease has led to the development of a new class of drugs to control platelet function. Inhibition of the final common pathway of platelet aggregation, with the glycoprotein IIb/IIIa receptor as a target, is a particularly logical strategy. The development of these drugs parallels the evolution of thrombolytic therapy a decade ago: the initial demonstration of their potency, the lack of understanding of a number of their mechanisms of action and nuances of their administration, and bleeding as the principal drawback to their use. Inhibitors of glycoprotein IIb/IIIa receptors are likely to be the first "anti-integrins" to be used widely,⁸¹ and they should lead to the development of other clinically useful agents directed against cellular adhesion molecules.

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